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The histone demethylase Kdm5 controls Hid-induced cell death in *Drosophila*

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We conducted an EMS mutagenesis screen on chromosome arm 2L to identify recessive suppressors of *GMR-hid*-induced apoptosis in the *Drosophila* eye. Through this screen, we recovered three alleles of the lysine demethylase gene *Kdm5*. Kdm5, a member of the JmjC-domain-containing protein family, possesses histone demethylase activity towards H3K4me3. Our data suggest that Kdm5 specifically regulates Hid-induced cell death during development, as we did not observe control of Reaper- or Grim-induced cell death by *Kdm5*. Interestingly, *GMR-hid*-induced apoptosis is suppressed independently of Kdm5's demethylase activity. Our findings indicate that Rbf and dMyc are necessary for *Kdm5* mosaics to suppress *GMR-hid*-induced cell death. Moreover, *Kdm5* mosaics failed to suppress apoptosis induced by a mutant form of Hid that is resistant to inhibition by Erk-type MAPK activity. Additionally, *Kdm5* dominantly enhances the wing phenotype of an activated MAPK mutant. These results collectively suggest that Kdm5 controls Hid-induced apoptosis by regulating the Rbf, dMyc, and MAPK pathways.

KEYWORDS

***Drosophila*, cell death, Hid, Kdm5, Rb, Myc, MAPK**

Introduction

Programmed cell death is an essential biological process during development and homeostasis of multi-cellular organisms. Apoptosis is the major form of cell death and accounts for more than 90% of all cell death (Newton et al., 2024). Apoptosis is evolutionarily conserved and is critical for the health of the organism. Alterations in the rate of apoptosis are linked to cancer, auto-immune diseases and neurodegeneration (Kayagaki et al., 2024). Therefore, a detailed understanding of the control of apoptosis is necessary to develop strategies for treatment of these diseases.

Control of apoptosis comes down to the control of caspases, a class of highly specialized Cys proteases which execute apoptosis when activated (Shalini et al., 2015). In living cells, caspases are inhibited by inhibitor-of-apoptosis proteins (IAPs) which directly bind to caspases and inhibit their activity (Gyrd-Hansen and Meier, 2010). In response to apoptosis-inducing signals, IAPs are degraded and release caspases for further activation. In *Drosophila*, the IAP-antagonists Reaper, Hid and Grim stimulate the ubiquitylation and degradation of *Drosophila* IAP1 (DIAP1) resulting in the release of the initiator caspase Dronc (Caspase-9-ortholog in *Drosophila*) from DIAP1 inhibition (Orme and Meier, 2009). Free Dronc is activated by incorporation into the apoptosome by the Apaf-1-like protein Dark (Dorstyn et al., 2018). Subsequently, the apoptosome activates

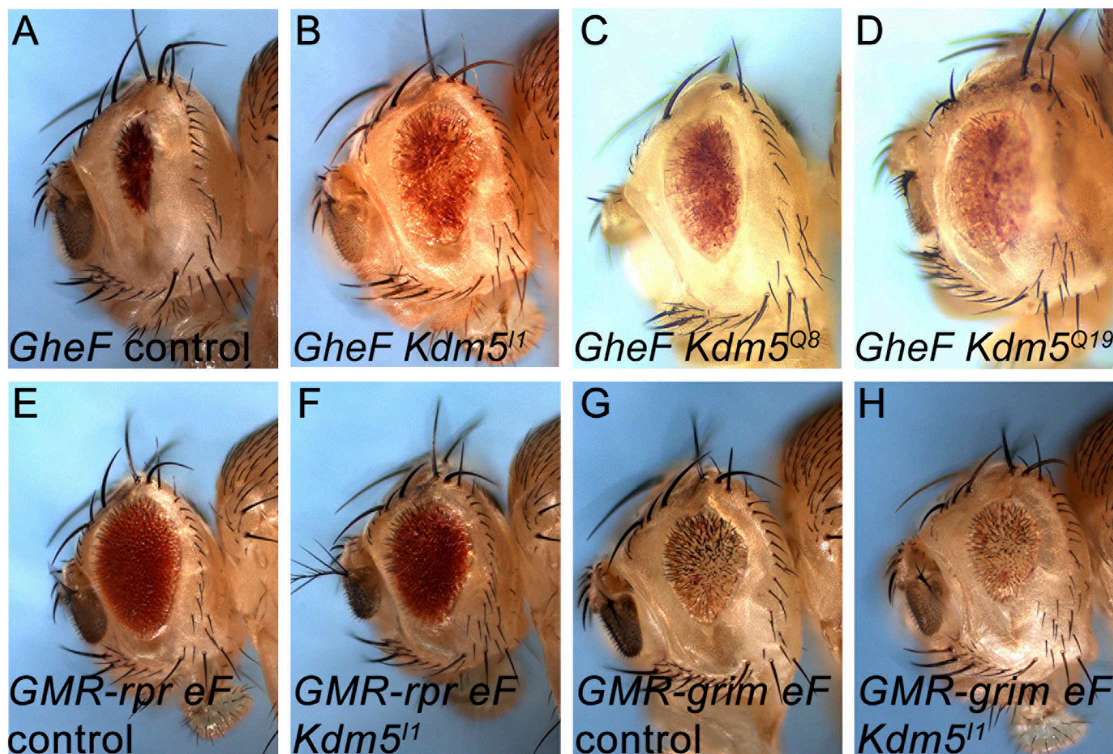

FIGURE 1
Identification of *Kdm5* mutants as recessive suppressors of *GMR-hid*. **(A)** The unmodified *GMR-hid ey-FLP* (*GheF*) eye ablation phenotype. **(B–D)** Three alleles of *Kdm5* behave as recessive suppressors of the *GheF* eye ablation phenotype in genetic mosaics. **(E–H)** *Kdm5* mutants do not suppress *GMR-reaper ey-FLP* (*GMR-rpr eF*) **(E, F)** and *GMR-grim ey-FLP* (*GMR-grim eF*) **(G, H)** eye ablation phenotypes in genetic mosaics. Genotypes: **(A)** *ey-FLP/+; FRT40/P[w+] FRT40; GMR-hid/+*. **(B)** *ey-FLP/+; Kdm5^{l1} FRT40/P[w+] FRT40; GMR-hid/+*. **(C)** *ey-FLP/+; Kdm5^{Q8} FRT40/P[w+] FRT40; GMR-hid/+*. **(D)** *ey-FLP/+; Kdm5^{Q19} FRT40/P[w+] FRT40; GMR-hid/+*. **(E)** *ey-FLP/+; ubi-GFP FRT40/CyO; GMR-rpr/+*. **(F)** *ey-FLP/+; Kdm5^{l1} FRT40/ubi-GFP FRT40; GMR-rpr/+*. **(G)** *ey-FLP/+; ubi GFP FRT40/CyO; GMR-grim/+*. **(H)** *ey-FLP/+; Kdm5^{l1} FRT40/ubi-GFP FRT40; GMR-grim/+*.

effector caspases such as DrICE and Dcp-1 (caspase-3-orthologs in *Drosophila*) (Kumar, 2007) which execute the apoptotic process.

Expression of the IAP-antagonist Hid in the *Drosophila* compound eye using the *GMR* promoter (*GMR-hid*) causes a strong eye ablation phenotype due to massive apoptosis induced by Hid in the eye imaginal disc during larval development (Figure 1A) (Fan and Bergmann, 2008; Grether et al., 1995). We have used the strong eye ablation phenotype of *GMR-hid* in chemical (EMS) mutagenesis screens to identify genes involved in the control of Hid-induced apoptosis. Initially, in dominant modifier screens of the *GMR-hid* eye ablation phenotype, heterozygous mutants of *diap1* (Goyal et al., 2000; Wilson et al., 2002) and of negative regulators of the EGFR/Ras/MAPK pathway such as *argos* and *sprouty* were recovered (Bergmann et al., 1998). Recovery of regulators of the EGFR/Ras/MAPK pathway revealed that Hid is negatively controlled both by MAPK phosphorylation and transcriptional repression (Bergmann et al., 1998; Bergmann et al., 2002; Kurada and White, 1998). However, mutations in the core components of the apoptotic pathway, *dronc*, *dark* and *drICE* were not recovered in the dominant/heterozygous modifier screens implying that they are not rate-limiting for the strong eye ablation phenotype of *GMR-hid* [reviewed in Xu et al. (2009)].

Therefore, in a second-generation mutagenesis effort, we screened homozygous mutants for suppression of *GMR-hid*. Because we assumed that mutants of the core apoptotic components would be homozygous lethal (which was later confirmed for *dronc* and *dark* mutations), we performed the mutagenesis screens in genetic mosaics obtained by *ey-FLP/FRT*-induced mitotic recombination (Newsome et al., 2000; Xu and Rubin, 1993). We referred to this procedure as *GheF* (*GMR-hid ey-FLP*) screening (Xu et al., 2005) (see Supplementary Figure S1). We performed *GheF* screening for all four autosomal chromosome arms (2L, 2R, 3L and 3R). Using *GheF* screening, we recovered mutations of *dronc* (located on 3L), *dark* (2R) and *drICE* (3R) as strong suppressors of the *GMR-hid*-induced eye ablation phenotype (Srivastava et al., 2007; Xu et al., 2005; Xu et al., 2006); reviewed in Xu et al. (2009). Furthermore, we recovered mutants in additional genes such as *D-cbl* (3L), *Uba1*, *vps25*, *ptc* and *cos2* (all 2R) as moderately strong suppressors of *GMR-hid* using *GheF* screening (Christiansen et al., 2012; Christiansen et al., 2013; Herz et al., 2006; Herz et al., 2009; Lee et al., 2008; Wang et al., 2008). These genes do not encode core components of the apoptotic pathway but can modify the outcome of the apoptotic process.

Here, we report the results of *GheF* screening for chromosome arm 2L. We recovered three mutant alleles of the gene *lysine demethylase 5* (*Kdm5*), also known as *little imaginal discs* (*lid*), as moderately strong suppressors of *GMR-hid* in genetic mosaics. *Kdm5* was initially identified as a Trithorax Group (TrxG) gene and encodes the *Drosophila* homolog of human Retinoblastoma-binding protein 2 (Rbp2) (Gildea et al., 2000). Rbp2 was identified as

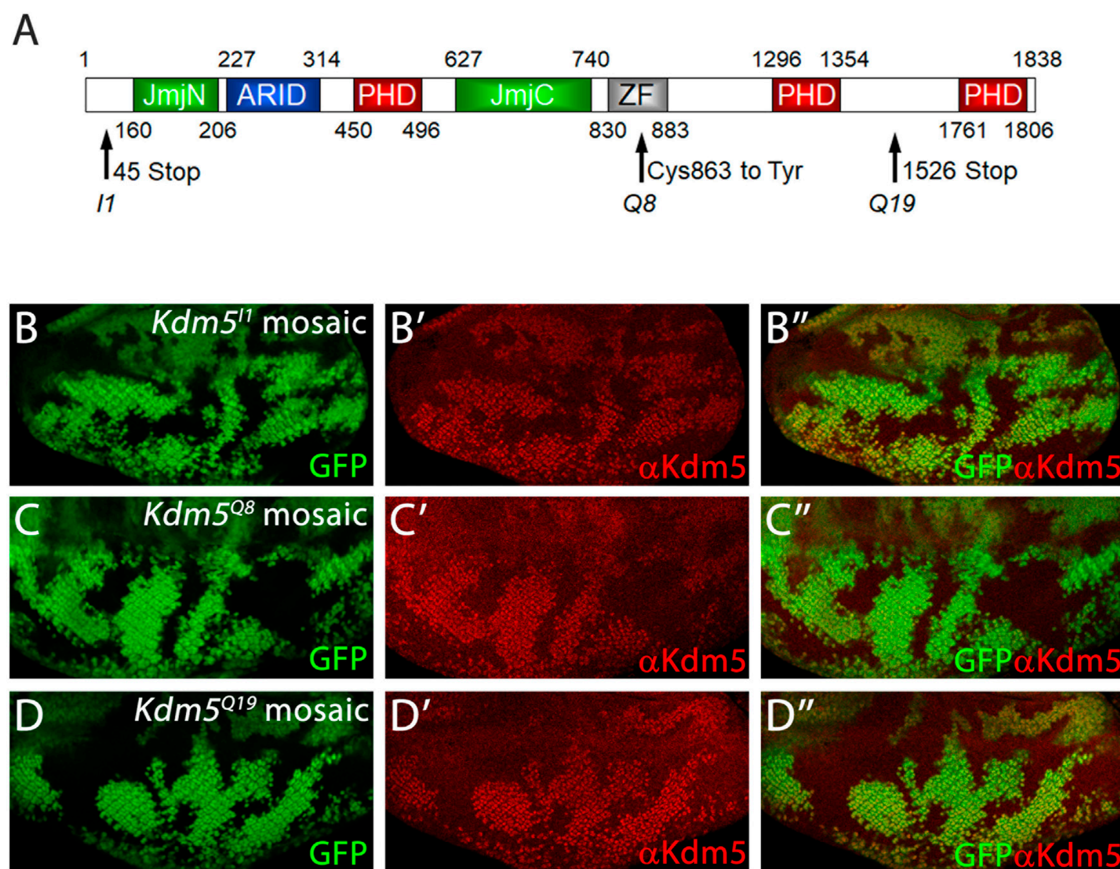

FIGURE 2
Mutant *Kdm5* genes encode unstable Kdm5 proteins. **(A)** Schematic outline of the protein domains of Kdm5. JmjN = JumonjiN, ARID = AT-rich interaction domain (DNA binding), PHD = Plant Homeo Domain (protein interaction), JmjC = JumonjiC, ZF = Zink Finger. Molecular lesions of $Kdm5^{I1}$, $Kdm5^{Q8}$ and $Kdm5^{Q19}$ are indicated by arrows. **(B–D)** Anti-Kdm5 antibody labeling of mosaic eye imaginal discs. Reduced Kdm5 protein levels are detected in *Kdm5*^{I1} **(B–B")**, *Kdm5*^{Q8} **(C–C")** and *Kdm5*^{Q19} **(D–D")** mutant clones (tissue). The absence of GFP identifies *Kdm5* mutant clones. Genotypes: *ey-FLP/+; Kdm5*^{x} *FRT40/ubi-GFP FRT40* with x = I1 **(B)**, Q8 **(C)** and Q19 **(D)**.

a Rb binding protein in a yeast two-hybrid screen and was shown to control Rb-mediated gene expression (Benevolenskaya et al., 2005; Defeo-Jones et al., 1991; Fattaey et al., 1993; Kim et al., 1994). After identification of Rbp2 as a JmjC-domain-containing histone demethylase, it was grouped as a member of the JARID1 family of histone demethylases (Klose et al., 2006). *Kdm5* is the only JARID1 ortholog in *Drosophila*, while the human genome contains four, with Rbp2 classified as JARID1A.

Kdm5 encodes a protein with multiple domains (Figure 2A). Most notable is the JmjC domain which has lysine demethylase activity and was found to specifically demethylate trimethylated lysine 4 of histone H3 (H3K4me3) (Eissenberg et al., 2007; Lee et al., 2007; Lloret-Llinares et al., 2008; Secombe et al., 2007). In addition, Kdm5 contains a JmjN domain, an ARID (A/T-rich interaction domain), a Zn finger (ZF) and three PHDs (plant homeobox domains) (Figure 2A). The ARID is implicated in binding to A/T-rich DNA sequences, while the ZF and the PHDs are involved in DNA/chromatin interactions. The JmjN, ARID, ZF and PHD1 domains are required for the demethylase activity of Kdm5 (Li et al., 2010). PHD3 can bind to H3K4me3 (Li et al., 2010; Liu and Secombe, 2015) while PHD1 binds to unmethylated Lys4 of histone 3 (H3K4me0) (Li et al., 2010; Torres et al., 2015).

H3K4me3 is enriched at promoter regions of transcriptionally active genes (Barski et al., 2007; Bernstein et al., 2005; Heintzman et al., 2007; Ng et al., 2003; Wirbelauer et al., 2005), and Kdm5 proteins were shown to colocalize with the H3K4me3 mark in those promoter regions (Liu and Secombe, 2015; Lloret-Llinares et al., 2012; Lopez-Bigas et al., 2008). Because the JmjC-domain of Kdm5 demethylates H3K4me3, a marker for gene activation, Kdm5 would be predicted to function as a silencer of gene expression by demethylating H3K4me3. However, this silencing function would be inconsistent with its original identification as a member of the TrxG gene family which is implicated in maintaining gene expression, for example, of homeotic genes. Consistently, Kdm5 has been shown to act as a transcriptional activator by maintaining *Ubx* expression in the haltere (Lloret-Llinares et al., 2008) and as co-activator of *Drosophila* Myc- (dMyc-) induced cell growth (Secombe et al., 2007). In the latter case, dMyc directly binds to the JmjC domain of Kdm5 and inhibits its catalytic demethylase activity. This enables the PHD3 of Kdm5 to bind to H3K4me3 and to recruit the Kdm5/dMyc complex to promoter regions of dMyc target genes such as Nop60B to stimulate dMyc-dependent transcription (Li et al., 2010; Liu and Secombe, 2015; Secombe et al., 2007). The interaction between

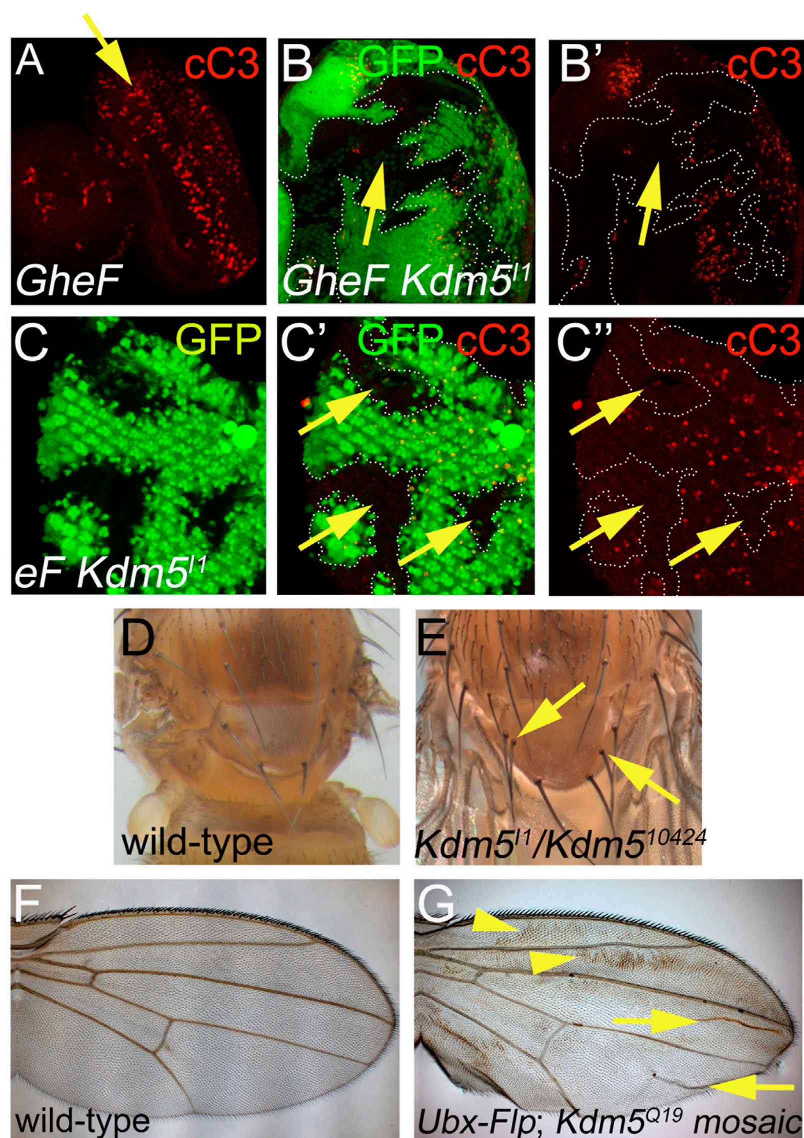

FIGURE 3
Cell death phenotypes of *Kdm5* mutants. **(A)** *GMR-hid* induced cell death in 3rd instar larval eye imaginal discs visualized by cleaved Caspase 3 (cC3) labeling (red). A yellow arrow points to a wave of apoptosis induced by *GMR-hid*. **(B, B')** *$Kdm5^{I1}$* mutant clones suppress *GMR-hid*-induced cell death in 3rd instar larval eye imaginal discs. White dotted lines outline the *Kdm5* mutant clones with decreased activity of cleaved Caspase3 (cC3). The yellow arrow points to an area of interrupted cC3 labeling in a *Kdm5* mutant clone. **(C–C'')** *$Kdm5^{I1}$* controls developmental cell death in pupal eye discs at 30 h after puparium formation (APF). *$Kdm5^{I1}$* mutant clones outlined by white dotted lines show decreased Caspase 3 (cC3) activity. The yellow arrows point to areas of absent cC3 labeling in *Kdm5* mutant clones. **(D)** Thorax of a wild-type fly with 4 scutellar bristles (macrochaetae). **(E)** Thorax of a *Kdm5* mutant fly with two additional macrochaetae (yellow arrows). Adult flies of genotype *$Kdm5^{I1}/Kdm5^{10424}$* are very rare. We recovered only 5 flies of this genotype. All five have duplications of thoracic macrochaetae bristles. **(F)** A wing of a wild-type fly. **(G)** A *Kdm5* mosaic wing displays a wing blemishing phenotype (yellow arrowheads) and additional wing vein material (yellow arrows). The penetrance of this phenotype is 100%. Genotypes: **(A)** *ey-FLP/+; FRT40/P[w+] FRT40; GMR-hid/+*. **(B)** *ey-FLP/+; $Kdm5^{I1}$ FRT40/P[w+] FRT40; GMR-hid/+*. **(C)** *ey-FLP/+; $Kdm5^{I1}$ FRT40/ubi-GFP FRT40.* **(D)** Canton S. **(E)** *$Kdm5^{I1}/Kdm5^{10424}$*. **(F)** Canton S. **(G)** *Ubx-FLP; $Kdm5^{Q19}$ FRT40/ubi-GFP FRT40.*

Kdm5 and dMyc is also evolutionarily conserved for the mammalian proteins (Secombe et al., 2007). Another mechanism by which Kdm5 can act as an activator of gene expression and thus as a TrxG protein is through inhibition of the histone deacetylase Rpd3 (Lee et al., 2009). Therefore, *Drosophila Kdm5* can act both as a transcriptional activator and transcriptional silencer. Consistent with this notion is also the observation that mammalian Rbp2/JARID1A can modulate both transcriptional activity and repression of Rb (Benevolenskaya et al., 2005). These opposite effects on control on transcription by Kdm5 are also confirmed by gene expression profiling analysis of *Kdm5* mutants which revealed that of the deregulated genes about half are downregulated while the other half are upregulated (Drelon et al., 2018; Liu et al., 2014; Liu and Secombe, 2015).

As demonstrated first for dMyc-induced cell growth (Secombe et al., 2007) and consistent with its role as transcriptional activator, many of Kdm5's functions are independent of the demethylase activity of the JmjC-domain of Kdm5 (Drelon et al., 2018; Hatch

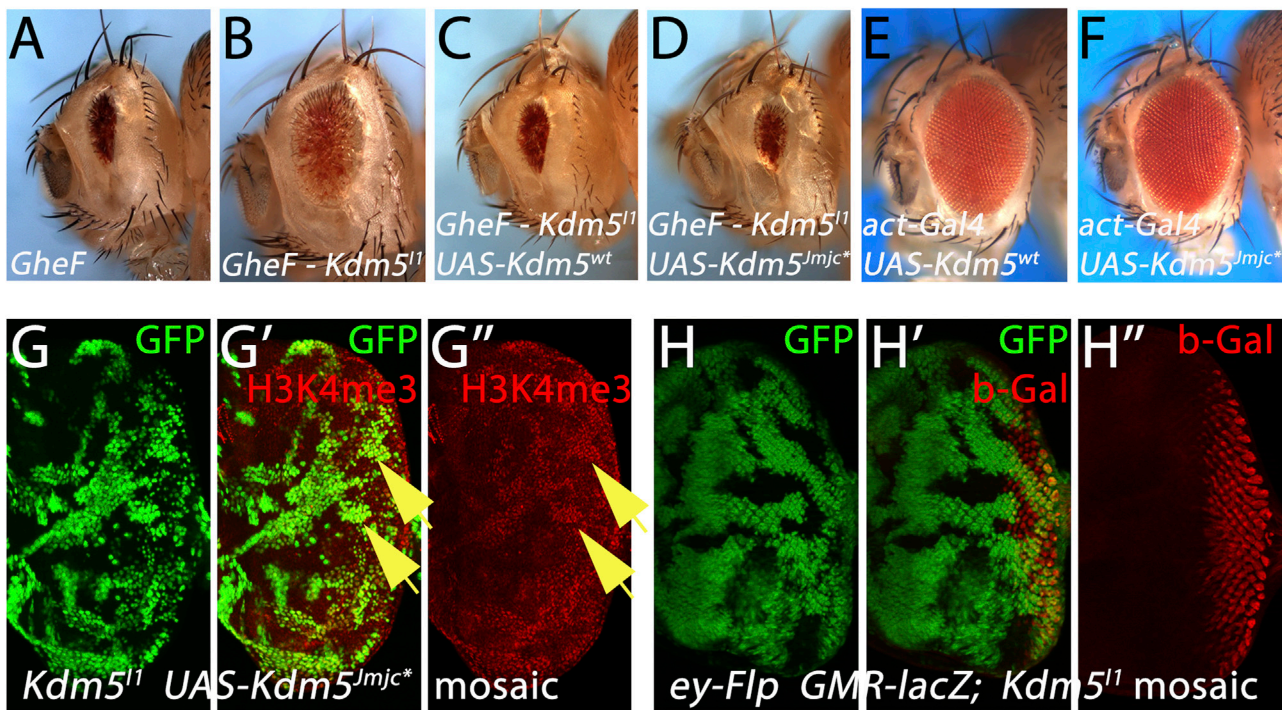

FIGURE 4
Kdm5 controls Hid-induced apoptosis independently of its JmjC demethylase activity. **(A–D)** Expression of UAS-transgenes encoding wild-type *Kdm5* (*UAS-Kdm5*^{wt}) **(C)** and a JmjC-domain mutant of Kdm5 (*UAS-Kdm5*^{JmjC}*) **(D)** can revert the suppression of the *GheF* eye ablation phenotype by *Kdm5*^{l1} mosaics **(B)** back to the original *GheF* eye ablation phenotype **(A)**. The *Kdm5*^{JmjC*} transgene mutates two invariant residues in the JmjC domain rendering the demethylase activity of Kdm5 inactive. **(E, F)** Expression of *UAS-Kdm5*^{wt} **(E)** and *UAS-Kdm5*^{JmjC*} **(F)** under control of *act-Gal4* (the same Gal4 driver used in **(C,D)**) does not cause an eye ablation phenotype. **(G–G")** The *UAS-Kdm5*^{JmjC*} transgene cannot rescue the enrichment of H3K4me3 marks in *Kdm5*^{l1} mutant clones (see yellow arrows as examples) and is thus catalytically inactive. In this experiment, *Kdm5*^{l1} mutant clones expressing *UAS-Kdm5*^{JmjC*} were induced using the MARCM method. Hence, mutant clones expressing *Kdm5*^{JmjC*} are positively marked by GFP. **(H–H")** *Kdm5* does not affect expression of transgenes from the *GMR* promoter. A *GMR-lacZ* reporter transgene does not show any changes of gene expression in *Kdm5*^{l1} mutant clones. Note that *Kdm5*^{l1} mutant clones are marked by absence of GFP in this experiment. Genotypes: **(A)** *ey-FLP/+; FRT40/P[w+] FRT40; GMR-hid/+*. **(B)** *ey-FLP/act-Gal4; Kdm5*^{l1} *FRT40/P[w+] FRT40; GMR-hid/+*. **(C)** *ey-FLP/act-Gal4; Kdm5*^{l1} *FRT40 UAS-Kdm5*^{wt}*/P[w+] FRT40; GMR-hid/+*. **(D)** *ey-FLP/act-Gal4; Kdm5*^{l1} *FRT40 UAS-Kdm5*^{JmjC*}*/P[w+] FRT40; GMR-hid/+*. **(E)** *act-Gal4; UAS-Kdm5*^{wt}. **(F)** *act-Gal4; UAS-Kdm5*^{JmjC*}. **(G)** *hs-FLP UAS-CD8:GFP; Kdm5*^{l1} *FRT40 UAS-Kdm5*^{JmjC*}*/P[tub-Gal80] FRT40; tub-Gal4/+*. **(H)** *ey-FLP GMR-lacZ/+; Kdm5*^{l1} *FRT40/ubi-GFP FRT40*.

et al., 2021; Liu et al., 2014; Liu and Secombe, 2015). In fact, the demethylase activity of Kdm5 is not essential for development of *Drosophila* animals (Li et al., 2010). Flies defective for the JmjC-demethylase function of Kdm5 are phenotypically normal, although males, but not females, are short lived (Li et al., 2010). The survival of these demethylase-deficient animals can be potentially explained by a redundancy with another H3K4 demethylase gene in *Drosophila*, *dKdm2*, but nevertheless, global H3K4me3 levels are increased in demethylase-deficient *Kdm5* flies (Li et al., 2010) suggesting that increased levels of H3K4me3 can be tolerated during development and also for normal survival of females. In contrast, a null allele of *Kdm5* is 100% lethal (Drelon et al., 2018) suggesting that *Kdm5* has essential functions during *Drosophila* development independently of its demethylase activity. The lethality of the null allele of *Kdm5* was attributed to its role in larval growth and was mostly due to reduced proliferation of imaginal discs resulting in pupal lethality (Drelon et al., 2018). Apoptosis was also found to be increased in *Kdm5* null mutant imaginal discs (Drelon et al., 2018), but the signal for control of apoptosis might be of non-autonomous origin coming from the prothoracic gland (Drelon et al., 2019). Kdm5 also regulates the expression of genes involved in mitochondrial morphology and physiology (Rogers et al., 2023) which is also a contributing factor for survival of the animals during development. Interestingly, restoring MAPK activity specifically in the prothoracic gland can restore viability in otherwise *Kdm5* null mutant animals (Rogers et al., 2023) suggesting that Kdm5 also controls MAPK activation.

Here, we report the recovery of three EMS-induced alleles of *Kdm5* as moderately strong suppressors of *GMR-hid*-induced eye ablation in genetic mosaics. Kdm5 specifically controls Hid-induced apoptosis and does not affect Reaper- and Grim-induced apoptosis. This function of Kdm5 is independent of the JmjC-demethylase activity. Furthermore, our data reveal that Kdm5 exerts its control over Hid-induced apoptosis through the activities of Rbf, dMyc, and MAPK. We present a model which incorporates these findings for the control of Hid-induced apoptosis.

Materials and methods

Identification of *Kdm5* mutant alleles by *GheF* screening

The EMS mutagenesis screen for suppressors of *GMR-hid* in *ey-FLP/FRT*-induced mosaics for chromosome arm 2L is outlined in

Supplementary Figure S1. Males of genotype *y w ey-FLP*; *y⁺ FRT40A* were incubated on tissue wipes soaked in 5% sucrose solution containing 25 mM Ethyl methanesulfonate (EMS) for 24 h. After that, they were allowed to recover for 3 h before being mated to virgin females of genotype *y w ey-FLP*; *w⁺ FRT40A/CyO*; *GMR-hid* at 25°C. 21,871 F1 offspring from this cross were screened for suppression of the *GMR-hid*-induced eye ablation phenotype. All dominant suppressors were discarded. 78 recessive suppressors were recovered, retested and balanced. One complementation group composed of three moderately strong suppressors of *GMR-hid* was identified by complementation crosses. Using several chromosomal deficiency stocks, the map position of this complementation group was determined at cytological interval 26A/B on chromosome arm 2L. Complementation crosses with existing *Kdm5* mutants $Kdm5^{10424}$ and $Kdm5^{k06801}$ (two P-element insertions in the first intron of the *Kdm5* gene (Gildea et al., 2000)) identified the newly isolated complementation group as mutants of *Kdm5*. This was further confirmed by sequencing, rescue crosses and phenotypic analysis.

Drosophila husbandry and genetics

All crosses were performed on standard cornmeal-molasses medium (60 g/L cornmeal, 60 mL/L molasses, 23.5 g/L baker's yeast, 6.5 g/L agar, 4 mL/L acid mix and 0.13% Tegosept). Genetic mosaics were induced in eye-antennal imaginal discs using the FLP/FRT mitotic recombination system (Xu and Rubin, 1993) using *ey-FLP* (Newsome et al., 2000) with GFP as genetic marker. Mutant clones are marked by loss of GFP. In the case of the MARCM (Lee and Luo, 2001) crosses in Figure 4G, mutant *Kdm5* clones expressing the $UAS\text{-}Kdm5^{JmjC*}$ transgene are positively marked by GFP. The wing in Figure 3G is taken from the F1 offspring of the following cross: *y w*; $Kdm5^{Q19}$ *FRT40/CyO* x *y w Ubx-FLP; w⁺ FRT40/CyO*. Non-*CyO* offspring was selected for imaging.

Immunohistochemistry

Eye-antennal imaginal discs from third instar larvae or 26–30 h APF pupal eye discs were dissected using standard protocols and labeled with antibodies raised against the following antigens: anti-Kdm5 (a kind gift of Julie Secombe); cleaved Caspase-3 (cC3) (Cell Signaling Technology); H3K4me3, H3K4me2, H3K4me1 (all Abcam); anti-Diap1 (a kind gift of H.D. Ryoo), anti-Dronc, anti-DrICE (all kind gifts of P. Meier); anti-Hid (a kind gift of H. Steller); anti-Rbf (a kind gift of N. Dyson); anti-β-Gal, anti-dMyc, anti-p53 (all DSHB); anti-JNK (Cell Signaling Technology). Cy3 fluorescently-conjugated secondary antibodies were obtained from Jackson ImmunoResearch. In each experiment, multiple clones in 10–20 eye imaginal discs were analyzed. Images were captured using an Olympus Optical FV500 confocal microscope.

Results

Isolation of three *Kdm5* alleles as recessive suppressors of *GMR-hid* in genetic mosaics

We performed an EMS mutagenesis screen on chromosome arm 2L to recover recessive suppressors of the *GMR-hid*-induced eye ablation phenotype in genetic mosaics obtained by *ey-FLP/FRT*-mediated mitotic recombination. The scheme of the *GheF* (*GMR-hid ey-FLP*) screen is shown in Supplementary Figure S1. We screened approximately 22,000 F1 offspring of EMS-treated males and *GheF*-bearing females, and recovered 78 suppressors in genetic mosaics. All of these suppressors are recessive as they do not suppress *GMR-hid* in a heterozygous, i.e., dominant manner.

By complementation analysis, we identified one complementation group consisting of three alleles, initially termed *I1*, *Q8* and *Q19*, which suppressed *GMR-hid* moderately strong in *ey-FLP/FRT*-induced mosaics (Figures 1A–D). Deficiency mapping with overlapping deficiencies covering chromosome arm 2L identified cytological interval 26B1/2 as the chromosomal location of this complementation group. Complementation crosses with existing mutants of genes in this chromosomal location identified *Kdm5* (formerly known as *little imaginal discs* (*lid*)) as the underlying gene of this complementation group. Consistently, a transgene encoding wild-type *Kdm5* can restore the strong eye ablation phenotype of *GMR-hid* in *Kdm5* mosaics (Figures 4A–C). Therefore, we refer to the newly isolated *Kdm5* alleles as $Kdm5^{I1}$, $Kdm5^{Q8}$ and $Kdm5^{Q19}$.

Interestingly, the eye ablation phenotype obtained by *GMR-reaper* and *GMR-grim* transgenes is not suppressed by *Kdm5* mosaics (Figures 1E–H). This observation suggests that Kdm5 specifically controls Hid-induced apoptosis.

Phenotypic characterization of the new *Kdm5* alleles

The three *Kdm5* alleles isolated in this study have point mutations in the residues indicated in Figure 2A. $Kdm5^{I1}$ has an early STOP codon at codon 45 (Figure 2A) and likely encodes a very strong, if not a null, loss-of-function mutant. Consistently, the suppression of *GMR-hid* by $Kdm5^{I1}$ is the strongest of the three alleles. Immunolabeling of mosaic $Kdm5^{I1}$ eye imaginal discs with anti-Kdm5 antibody confirms that no Kdm5 protein is produced in $Kdm5^{I1}$ mutant clones (Figures 2B, B'). $Kdm5^{Q8}$ has a point mutation changing Cys863 in the ZF domain to Tyr, and Kdm^{Q19} has a premature STOP codon at codon 1,526 (Figure 2A). Using the anti-Kdm5-specific antibody, we found that the mutant $Kdm5^{Q8}$ and $Kdm5^{Q19}$ genes encode unstable proteins (Figures 2C, D'). In the case of $Kdm5^{Q19}$, this may be due to nonsense-mediated RNA decay, while the point mutation in the ZF domain of $Kdm5^{Q8}$ appears to render the protein unstable.

Previous work has shown that the JmjC-demethylase domain of Kdm5 demethylates H3K4me3 (Eissenberg et al., 2007; Lee et al., 2007; Lloret-Llinares et al., 2008; Secombe et al., 2007). Using our newly generated *Kdm5* alleles, we confirmed that the global levels of H3K4me3 are increased in *Kdm5* mutant clones of larval mosaic eye-antennal imaginal discs (Supplementary Figures S2A–A'') suggesting that Kdm5 indeed possesses H3K4me3 demethylase activity and further confirming that the newly recovered alleles are *Kdm5* mutants. Consistent with previous observations (Eissenberg et al., 2007; Lee et al., 2007; Lloret-Llinares et al., 2008; Secombe et al., 2007), our genetic analysis of *Kdm5* mosaics does not demonstrate any demethylase activity towards H3K4me2 and H3K4me1 (Supplementary Figures S2B–C''). These

TABLE 1 Gene expression analysis of apoptotic genes in *Kdm5* mutant clones. Listed are proteins and reporter genes which are directly or indirectly involved in the control of apoptosis. With the exception of cleaved Caspase 3, none of the proteins or reporter genes are deregulated in *Kdm5* mutant clones in otherwise wild-type background in eye imaginal discs from 3rd instar larvae. Cleaved Caspase 3 antibody labeling was reduced in pupal eye discs at 26–30 h after puparium formation (APF) (see Figure 3C). The genotype used for the determination of the protein levels in the first and second columns was *ey-FLP/+; Kdm5^{I1} FRT40/ubi-GFP FRT40.* The genotype in the third and fourth column was *ey-FLP/+; Kdm5^{I1} FRT40/ubi-GFP FRT40* plus the reporter transgene indicated.

Protein	Change of protein levels	Reporter	Change of reporter expression
cleaved Caspase 3	down in clones (30 h APF)	*dark-lacZ*	—
DIAP1	—	*bantam sensor*	—
DrICE	—	*diap1-lacZ*	—
Dronc	—		
Hid	—		
JNK	—		
dMyc	—		
Rbf1	—		
p53	—		

findings confirm that *Kdm5* encodes a H3K4me3 demethylase consistent with previous reports (Eissenberg et al., 2007; Lee et al., 2007; Lloret-Llinares et al., 2008; Secombe et al., 2007).

Cell death phenotypes of *Kdm5* mutants

Because we identified the *Kdm5* alleles as suppressors of the *GMR-hid*-induced eye ablation phenotype which is caused by massive apoptosis in the larval eye imaginal disc (Figure 3A (Fan and Bergmann, 2008; 2014), we examined whether *Kdm5* affects apoptosis. First, the *GMR-hid*-induced apoptosis in larval eye imaginal discs is strongly suppressed autonomously in *Kdm5* mutant clones (Figures 3B, B'), explaining the recovery of eye tissue in *GMR-hid/Kdm5* mosaic flies.

We also examined whether *Kdm5* has a role for normal developmentally occurring programmed cell death. In the pupal retina, all unspecified cells are removed by Hid-dependent apoptosis (Kurada and White, 1998). In *Kdm5* mutant clones, this developmental apoptosis is strongly reduced (Figure 3C). Furthermore, while wild-type flies contain 4 scutellar bristles (macrochaetae) on the scutellum (Figure 3D), homozygous hypomorphic *Kdm5^{I1}/Kdm5^{10424}* survivors carry 1 to 2 additional macrochaetae (Figure 3E), a phenotype associated with reduced apoptosis (Kanuka et al., 2005) and also observed for *dronc*, *dark* and *cytochrome c* mutants (Lindblad et al., 2021; Mendes et al., 2006; Rodriguez et al., 1999). Finally, a wave of apoptosis occurs during wing maturation in newly eclosed flies (Kimura et al., 2004). When this apoptosis is blocked, a wing blemishing phenotype appears due to incomplete apoptosis as observed for *H99* (deleting *reaper*, *hid* and *grim*), *dronc*, *dark* and *drICE* mutants (Chew et al., 2004; Link et al., 2007). In *Kdm5* mosaic wings, a wing blemishing phenotype occurs in all animals tested (Figures 3F, G). Together, these data illustrate that *Kdm5* is an important gene for the control of apoptosis during *Drosophila* development.

Kdm5 controls apoptosis independently of its demethylase activity

Although Kdm5 is not a transcription factor, its best characterized function is control of transcription, either as an activator or as a silencer (Secombe and Eisenman, 2007). The distinguishing feature between these two opposing functions is the requirement of the demethylase activity of the JmjC domain of Kdm5 (Secombe and Eisenman, 2007). Therefore, we examined if the JmjC demethylase activity of Kdm5 is required for the control of apoptosis. As expected, expression of a *UAS-Kdm5^{wt}* rescue transgene can revert the suppression of *GMR-hid* by *Kdm5* mosaics (Figures 4A–C). Interestingly, a *UAS-Kdm5^{JmjC*}* transgene in which two critical residues in the demethylase domain have been mutated and thus generate a catalytic mutant (Secombe et al., 2007), can also revert the suppression of *GMR-hid* by *Kdm5* mosaics (Figure 4D) suggesting that the JmjC demethylase activity of Kdm5 is not required for the suppression of *GMR-hid*. Expression of the *UAS-Kdm5^{wt}* and *UAS-Kdm5^{JmjC*}* transgenes under control of *act-Gal4* [the same Gal4 driver used in Figures 4C, D)] does not cause an eye ablation phenotype (Figures 4E, F). This suggests that the reversal of *GMR-hid* suppression by *Kdm5* mosaics observed with these UAS transgenes (Figures 4C, D) is not due to eye ablation caused by the expression of the *UAS-Kdm5^{wt}* and *UAS-Kdm5^{JmjC*}* alone.

To verify that the *UAS-Kdm5^{JmjC*}* transgene indeed encodes a demethylase-defective variant, we found that the elevated H3K4me3 levels in *Kdm5* mutant clones are not normalized by expression of the *UAS-Kdm5^{JmjC*}* transgene (Figure 4G”) confirming that the *Kdm5^{JmjC*}* transgene is indeed demethylase-deficient. These results suggest that Kdm5 controls Hid-induced apoptosis independently of its demethylase activity. The demethylase-independent function of Kdm5 implies that it may act as a transcriptional activator for control of Hid-induced apoptosis.

Kdm5 does not control the expression of apoptotic genes

To examine the mechanism by which Kdm5 controls Hid-induced apoptosis, we tested the expression levels of genes involved in apoptosis. First, although we already found that *Kdm5* mosaics do not affect the *GMR-reaper*- and *GMR-grim*-induced eye ablation phenotypes (Figure 1), we nevertheless tested whether Kdm5 can act directly on the *GMR* promoter and thus controls *hid* expression from *GMR-hid*. However, expression of a *GMR-lacZ* reporter transgene is not affected in *Kdm5* mosaics (Figure 4H") suggesting that Kdm5 does not transcriptionally control the *GMR* promoter. Furthermore, the levels of all genes involved in and required for apoptosis (*dronc*, *dark*, *drICE*, *hid*, etc.) were not changed in *Kdm5* mutant clones (Table 1).

Although the lack of a requirement of the demethylase domain indicates an activator function of Kdm5 for control of Hid-induced apoptosis, we also examined genes encoding negative regulators of apoptosis whose deregulation would require a silencing function of Kdm5 to suppress *GMR-hid* when *Kdm5* is mutant. However, DIAP1 protein levels and expression of β-Gal from a *diap1-lacZ* reporter transgene are normal in *Kdm5* mutant clones (Table 1). A Hid-specific inhibitor of apoptosis is the miRNA *bantam*, targeting the 3′UTR of the *hid* mRNA (Brennecke et al., 2003). However, a *bantam* sensor is unchanged in *Kdm5* mutant clones (Table 1). These data suggest that Kdm5 is not directly controlling the gene expression or protein levels of apoptotic genes for control of Hid-induced apoptosis.

Kdm5 controls hid-induced cell death through Rbf and dMyc

To gain further insight into the role of Kdm5 for the control of Hid-induced apoptosis in *Drosophila*, we considered that the mammalian homolog of Kdm5, Rbp2 (JARID1A), was identified as a Rb binding protein (Defeo-Jones et al., 1991; Fattaey et al., 1993; Kim et al., 1994). The *Drosophila* homolog of Rb, Rbf, has been implicated in the control of Hid-induced apoptosis through inhibition of E2F1-dependent transcription (Moon et al., 2006). Therefore, we tested for a genetic interaction between *Rbf* and the ability of *Kdm5* mosaics to suppress the *GMR-hid* eye ablation phenotype (Figures 5A, B). Indeed, heterozygosity of *Rbf* using two different *Rbf* alleles can revert the suppression of *GMR-hid* by *Kdm5* mosaics back to the unmodified eye ablation phenotype (Figures 5A–D) suggesting that the suppression of the *GMR-hid* eye ablation phenotype by *Kdm5* mosaics requires *Rbf*. The *Rbf* mutants to not modify the *GMR-hid* eye ablation phenotype on their own (i.e., in a *Kdm5* wild-type background) (Figure 5H) suggesting that *Rbf* is rate-limiting for the suppression of *GMR-hid* by *Kdm5*. One function of Rbf is to negatively control the transcription factor E2F1 (Moon et al., 2006). Therefore, we also tested for a genetic interaction between *E2F1* and *Kdm5*. Consistently, the suppressed eye ablation phenotype of *GMR-hid* by *Kdm5* is even more strongly suppressed if *E2F1* is heterozygous in this genetic background (Figure 5E). Heterozygous *E2F1* mutants alone do not modify the *GMR-hid* phenotype (Figure 5I). These genetic interactions suggest that Kdm5 controls *GMR-hid*-induced apoptosis through negative regulation of Rbf.

Another factor which was shown to interact with Kdm5 both genetically and physically is *Drosophila* Myc (dMyc) (Secombe et al., 2007). In this context, it is interesting to note that this interaction results in inhibition of the demethylase activity of Kdm5 (Secombe et al., 2007) which is not required for the suppression of *GMR-hid* (Figure 4D). Therefore, we performed similar genetic interaction experiments as with *Rbf* and *E2F1*. Indeed, heterozygously, *dMyc* mutants revert the suppression of *GMR-hid* by *Kdm5* back to the original eye ablation phenotype (Figures 5F, G) suggesting that *dMyc* is required for the suppression of *GMR-hid* by *Kdm5*. Heterozygous *dMyc* mutants alone do not suppress the *GMR-hid* eye ablation phenotype (Figure 5J) suggesting that *dMyc* is rate-limiting for the suppression of *GMR-hid* by *Kdm5*. This genetic interaction implies that Kdm5 and dMyc have an antagonistic relationship in this context.

Finally, to examine if Rbf/E2F1 and dMyc cooperate for the control of *GMR-hid* induced cell death, we took advantage of the observation that overexpression of *dMyc* can suppress the *GMR-hid* eye ablation phenotype (Figures 5K, L). This suppression is reverted back to the original *GMR-hid* eye ablation phenotype by heterozygosity of *Rbf* (Figure 5M) suggesting that *dMyc* requires *Rbf* to suppress *GMR-hid*. Together, these genetic interaction data demonstrate that *Kdm5* regulates *GMR-hid* through inhibition of *Rbf* and *dMyc*, and that *Rbf* and *dMyc* cooperate in this context.

Kdm5 regulates *GMR-hid*-induced eye ablation through control of MAPK signaling

While E2F1 and potentially dMyc can directly control endogenous *hid* expression (Moon et al., 2005), this type of regulation does not explain the suppression of *GMR-hid* by *Kdm5* as *hid* expression from *GMR-hid* is independent of the control of endogenous *hid*. This consideration would imply a post-translational control of Hid directly or indirectly by Kdm5. We have previously shown that Erk-type MAPK, encoded by the *rolled* (*rl*) gene, can post-translationally control Hid function by inhibitory phosphorylation (Bergmann et al., 1998). This type of regulation by MAPK on *GMR-hid* would also explain the inability of *Kdm5* mosaics to suppress *GMR-reaper* and *GMR-grim* (Figure 1) which are not subject to control by MAPK (Bergmann et al., 1998; Kurada and White, 1998).

Therefore, we examined if *Kdm5* mosaics can suppress a *GMR-hid* mutant in which the five MAPK phosphorylation sites of Hid have been mutated ($GMR\text{-}hid^{Ala5}$) and is thus unresponsive to inhibitory MAPK phosphorylation (Bergmann et al., 1998). Indeed, *Kdm5* mosaics are not able to suppress the eye ablation phenotype of $GMR\text{-}hid^{Ala5}$ (Figures 6A–C). As a positive control, to demonstrate that the very strong $GMR\text{-}hid^{Ala5}$ eye ablation phenotype is in fact suppressible, we tested another mutant, *S2*, which was recovered in the same *GheF* screen of chromosome arm 2L and which displays a similar moderate suppression of $GMR\text{-}hid^{wt}$ in mosaics as *Kdm5* (Supplementary Figure S1). *S2* is a single allele recovered in the *GheF* 2L screen and the underlying gene mutated in *S2* is unknown. Indeed, *S2* mosaics can suppress the $GMR\text{-}hid^{Ala5}$ eye ablation phenotype (Figure 6D) suggesting that the $GMR\text{-}hid^{Ala5}$ eye

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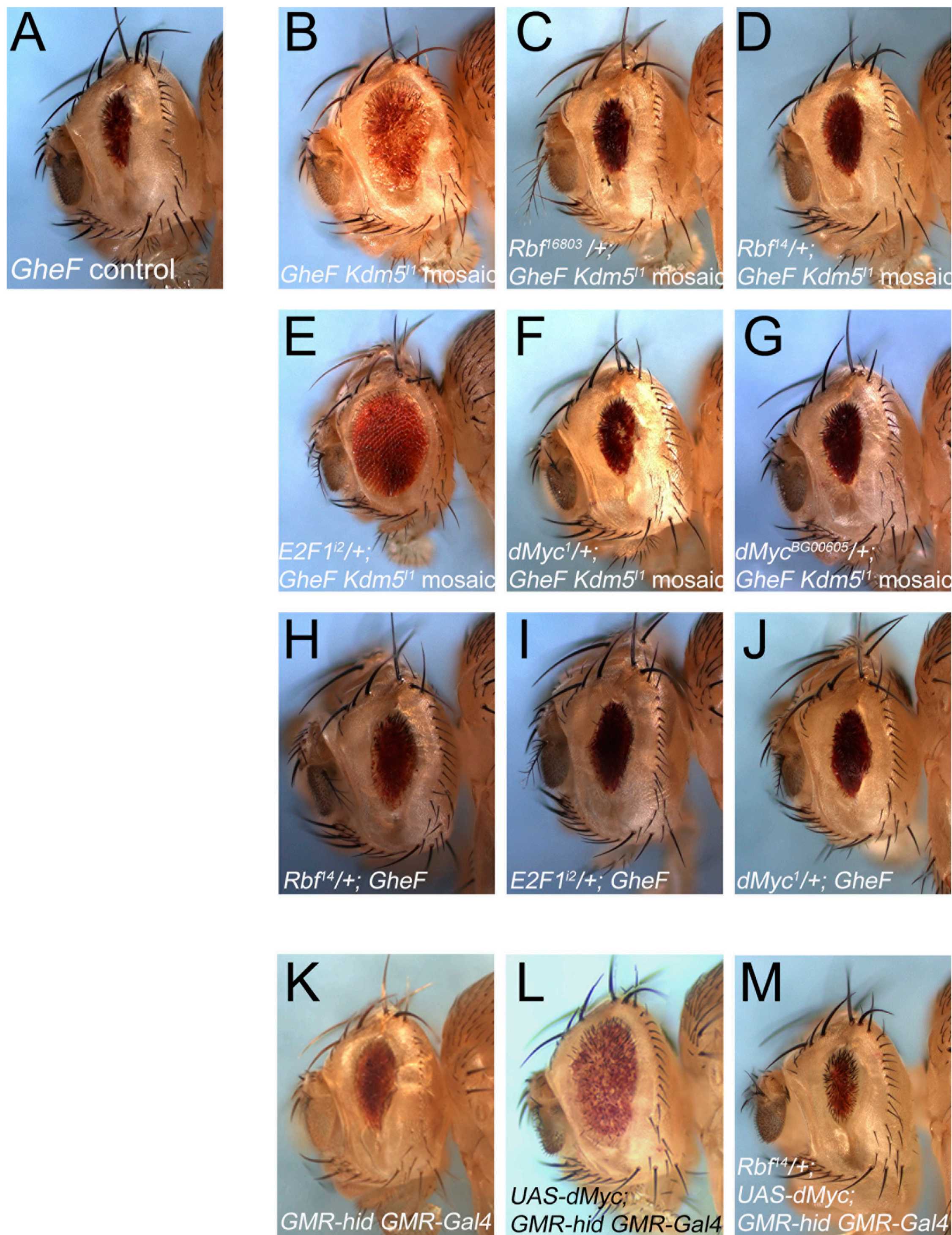

FIGURE 5
Kdm5 controls Hid-induced apoptosis through Rbf/E2F1 and dMyc. **(A)** The unmodified *GheF* eye ablation phenotype. **(B)** *Kdm5^{l1}* mosaics suppress the *GheF* eye ablation phenotype. **(C, D)** Two independent *Rbf* mutant alleles can dominantly revert the suppression of *GheF*-induced eye ablation by *Kdm5^{l1}* mosaics back to the original *GheF* eye ablation phenotype (compare to (A)) with 100% penetrance. **(E)** An *E2F1* mutant allele dominantly suppresses even further the *GheF* eye ablation phenotype by *Kdm5^{l1}* mosaics (compare to (B)) with 100% penetrance. **(F, G)** Two independent *dMyc* mutants can dominantly revert the suppression of *GheF*-induced eye ablation by *Kdm5^{l1}* mosaics back to the original *GheF* eye ablation phenotype (compare to (A)) with 100% penetrance. **(H–J)** The *Rbf^{14}*, *E2F1^{i2}* and *dMyc^{1}* mutants used in panels **(D–F)** do not modify the *GheF* eye ablation phenotype. **(K)** The unmodified *GMR-hid GMR-Gal4* eye ablation phenotype. **(L)** Expression of *UAS-dMyc* by *GMR-Gal4* suppresses the *GMR-hid* eye ablation phenotype. **(M)** *Rbf^{14}* can dominantly revert the suppression of *GMR-hid* by *dMyc* overexpression back to the original *GMR-hid* eye ablation phenotype

(*Continued*)

FIGURE 5 (Continued)
with 100% penetrance. Genotypes: **(A)** *ey-FLP/+; FRT40/P[w+] FRT40; GMR-hid/+*. **(B)** *ey-FLP/+; Kdm5^{I1} FRT40/P[w+] FRT40; GMR-hid/+*. **(C)** *ey-FLP/Rbf^{16803}; Kdm5^{I1} FRT40/P[w+] FRT40; GMR-hid/+*. **(D)** *ey-FLP/Rbf^{14}; Kdm5^{I1} FRT40/P[w+] FRT40; GMR-hid/+*. **(E)** *ey-FLP/+; Kdm5^{I1} FRT40/P[w+] FRT40; GMR-hid/E2F1^{i2}*. **(F)** *ey-FLP/dMyc^{1}; Kdm5^{I1}FRT40/P[w+] FRT40; GMR-hid/+*. **(G)** *ey-FLP/dMyc^{BG00605}; Kdm5^{I1}FRT40/P[w+] FRT40; GMR-hid/+*. **(H)** *ey-FLP/Rbf^{14}; P[w+] FRT40/+; GMR-hid/+*. **(I)** *ey-FLP/+; P[w+] FRT40/+; GMR-hid/E2F1^{i2}*. **(J)** *ey-FLP/dMyc^{1}; GMR-hid/+*. **(K)** *GMR-hid GMR-Gal4/+*. **(L)** *GMR-hid GMR-Gal4/UAS-dMyc*. **(M)** *Rbf^{14}/+; GMR-hid GMR-Gal4/UAS-dMyc*.

ablation phenotype is suppressible and that the inability of *Kdm5* to suppress *GMR-hid^{Ala5}* might be because of its unresponsive nature to MAPK phosphorylation. The MAPK-dependent suppression of *GMR-hid^{wt}* by *Kdm5* suggests that Kdm5 negatively regulates MAPK activity and therefore that *Kdm5* mutants have increased MAPK activity.

To further characterize the genetic interaction between *rl/MAPK* and *Kdm5*, we took advantage of the wing vein phenotype caused by a weak gain-of-function mutant of MAPK called *rl^{Sevenmaker}* (*rl^{Sem}*) (Brunner et al., 1994) (Figures 6E, F). The wing vein phenotype of *rl^{Sem}* is strongly enhanced by heterozygosity of *Kdm5* (Figure 6H) further supporting that Kdm5 negatively regulates MAPK. In strong cases, we also observed a wing blistering phenotype which was not observed in *rl^{Sem}* wings alone (Figure 6I). Heterozygous *Kdm5* wings do not show any specific wing vein phenotype (data not shown). Interestingly, heterozygosity of *hid* also strongly enhances the *rl^{Sem}* wing vein phenotype (Figure 6G) demonstrating the strong genetic link between *Kdm5* and *hid* through MAPK signaling. In summary, these genetic interaction studies demonstrate that Kdm5 regulates Hid through control of MAPK activity.

Discussion

In this paper, we report the recovery of three alleles of the histone demethylase gene *Kdm5* as moderately strong suppressors of *GMR-hid*-induced apoptosis. To our knowledge, these are the first EMS-induced alleles of *Kdm5*. We identified the mutations of these alleles and found that they either produce truncated proteins or unstable proteins, or both (Figure 2). Kdm5 is a nuclear protein and although it is not a transcription factor, all evidence points to an essential role of Kdm5 in transcriptional control, both as an activator and a silencer. The silencing function of Kdm5 is mediated through demethylation of H3K4me3 by the JmjC domain (Secombe and Eisenman, 2007). However, the demethylase activity of the JmjC domain of Kdm5 is not involved in the control of Hid-induced apoptosis (Figure 4D). Therefore, other domains of Kdm5 are involved in the control of Hid-induced apoptosis. A good candidate for such a domain is the ZF domain, as the *Kdm5^{Q8}* allele changes a conserved Cys residue to Tyr. However, this mutation causes protein instability (Figure 2) and the general instability of Kdm5^{Q8} protein might cause the suppression, and not the specific mutation in the ZF domain.

The control of apoptosis is very specific to the IAP antagonist Hid, as both the *GMR-reaper*- and *GMR-grim*-induced eye ablation phenotypes are not affected by *Kdm5* mosaics (Figure 1). Consistently, Kdm5 does not control expression of transgenes from the *GMR* promoter and of core components of the apoptotic pathway such as *dronc*, *dark* and *diap1* which would otherwise also affect Reaper- and Grim-induced apoptosis. The phenotypic characterization of the new *Kdm5* alleles supports the notion that Kdm5 is at least partially required for apoptosis. Consistently, it was reported that overexpression of *Kdm5* can ectopically induce apoptosis in a *dMyc*-expressing context (Secombe et al., 2007) and that *JARID1A$^{-/-}$* mice show a decrease in apoptosis of hematopoietic stem cells (Klose et al., 2007). However, it has also been reported that wing imaginal discs of homozygous *Kdm5* mutant larvae have actually increased levels of apoptosis (Drelon et al., 2018). Nevertheless, this increased apoptosis in wing imaginal discs is caused non-autonomously by signaling defects in the mutant prothoracic gland (Drelon et al., 2019). We would also like to emphasize that Kdm5 only controls Hid-induced apoptosis. Thus, other types of apoptosis, such as Reaper- or Grim-induced apoptosis which are not under Kdm5 control, may be the cause of this ectopic apoptosis seen in *Kdm5* mutants.

While we were unable to identify a specific target gene of Kdm5 that is involved in the control of apoptosis (see Table 1), we established genetic interactions between Kdm5 on one hand and Rbf/E2F1 and dMyc on the other hand for the control of Hid-induced apoptosis (Figure 5). The mammalian ortholog of *Drosophila* Kdm5, Rbp2/JARID1A was originally identified as an interacting protein with Rb, and that interaction is conserved in *Drosophila* (Benevolenskaya et al., 2005; Defeo-Jones et al., 1991; Fattaey et al., 1993; Kim et al., 1994; Secombe et al., 2007). Both *Drosophila* Kdm5 and mammalian JARID1A can form a protein complex with the corresponding Myc orthologs, which in *Drosophila* controls Myc-induced growth (Drelon et al., 2018; Secombe et al., 2007). Interestingly, dMyc binds to the JmjC domain of Kdm5 and inhibits its demethylase activity (Secombe et al., 2007). This inhibition converts the silencer function of Kdm5 into a transcriptional (co-) activator (Secombe and Eisenman, 2007; Secombe et al., 2007). Given that the demethylase function of Kdm5 is not required for the control of Hid-induced apoptosis (Figure 4) and that dMyc is rate-limiting for the control of Hid-induced apoptosis by Kdm5 (Figure 5), it is possible that a similar inhibition of Kdm5's demethylase activity by dMyc occurs for the control of Hid-induced apoptosis by Kdm5.

Putting all these data together, we propose the following model of Kdm5 action for the control of Hid-induced apoptosis. Kdm5 forms protein complexes with both Rbf and dMyc (Figure 7). dMyc inhibits the demethylase function of Kdm5 and thus maintains high levels of H3K4me3 at promoter regions of dMyc target genes. At the same time, the interaction between Rbf and Kdm5 blocks Rbf's ability to inhibit E2F1 which can now also bind to enhancer/promoter regions of target genes (Figure 7). Our genetic analysis suggests that dMyc and E2F1 synergistically control Hid-

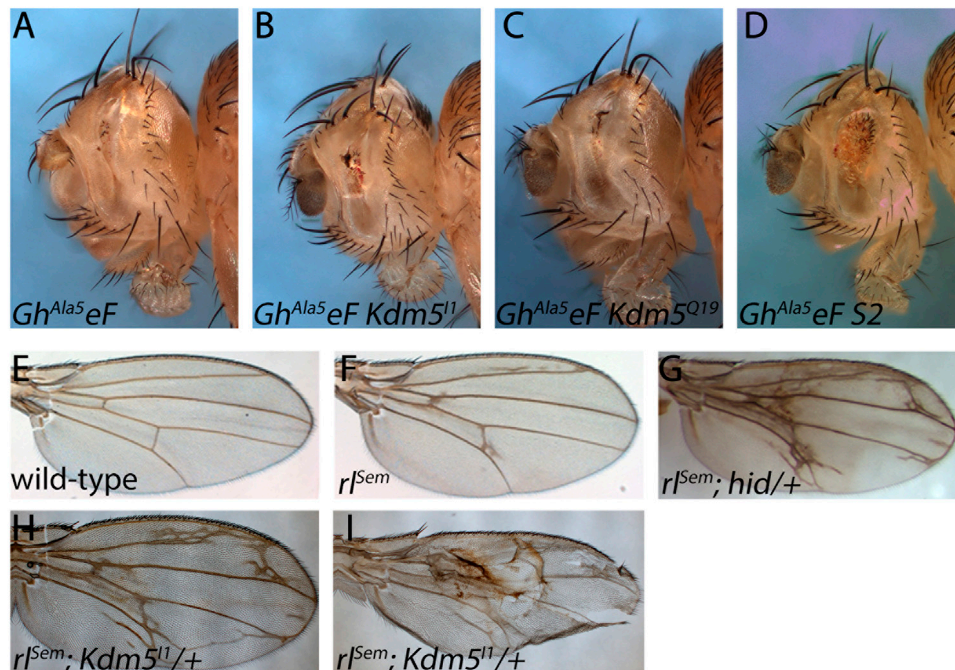

FIGURE 6
The suppression of *GMR-hid* by *Kdm5* is sensitive to MAPK activity. **(A)** A *GMR-hid* transgene in which the five MAPK phosphorylation sites of Hid have been mutated to unphosphorylatable Ala residues (*GMR-hid*^{Ala5}) and is unresponsive to MAPK phosphorylation causes a very strong eye ablation phenotype. *Gh*^{Ala5}*eF* = *GMR-hid*^{Ala5} *ey-FLP*. **(B, C)** *Kdm5* mosaics of *Kdm5*^{I1} and *Kdm5*^{Q19} are unable to suppress the *GMR-hid*^{Ala5} eye ablation phenotype. **(D)** An unrelated suppressor of *GMR-hid*, *S2* (see Supplementary Figure S1) is able to suppress *GMR-hid*^{Ala5} in genetic mosaics. **(E)** A wing of a wild-type fly. **(F)** A wing of a heterozygous gain-of-function mutant of MAPK, encoded by the *rolled* gene, *rolled*^{Sevenmaker} (*rl*^{Sem}/+). **(G)** Heterozygous *hid* mutants dominantly enhance the *rl*^{Sem} wing phenotype. Note the extra wing vein material. **(H, I)** Heterozygosity of *Kdm5* dominantly enhances the *rl*^{Sem} wing phenotype with a lot of extra wing vein material similar to *rl*^{Sem}/+; *hid*/+ **(G)**. In extreme cases, wing blistering is observed **(I)**. Genotypes: **(A)** *GMR-hid*^{Ala5} *ey-FLP; ubi-GFP FRT40/CyO*. **(B)** *GMR-hid*^{Ala5} *ey-FLP; Kdm5*^{I1} *FRT40/ubi-GFP FRT40*. **(C)** *GMR-hid*^{Ala5} *ey-FLP; Kdm5*^{Q19} *FRT40/ubi-GFP FRT40*. **(D)** *GMR-hid*^{Ala5} *ey-FLP; S2 FRT40/ubi-GFP FRT40*. **(E)** Canton S. **(F)** *rl*^{Sem}/+. **(G)** *rl*^{Sem}/+; *hid*$^{WR+X1}$/+. **(H, I)** *rl*^{Sem}/*Kdm5*^{I1}.

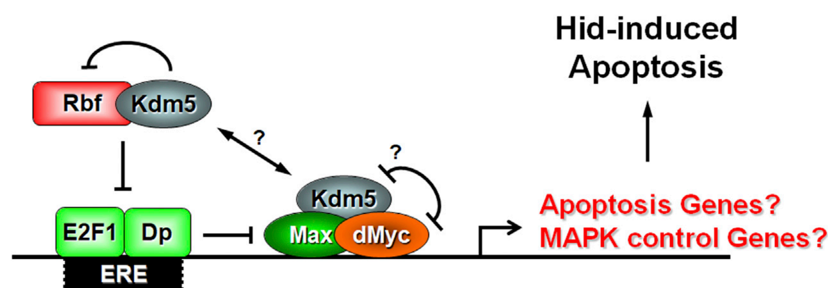

FIGURE 7
Model of Kdm5's function for control of the apoptotic activity of Hid. Our genetic interaction data and previously published biochemical data suggest that Kdm5 interacts with both Rbf and dMyc. The interaction between Kdm5 and Rbf is inhibitory. The genetic interaction between *Kdm5* and *dMyc* reveals a potential mutually inhibitory relationship, although it is unclear if dMyc directly inhibits the JmjC-dependent histone demethylase activity of Kdm5 as it does in another context (Secombe et al., 2007). Our genetic interaction studies also indicate an inhibitory relationship between E2F1 and dMyc for the control of Hid-induced apoptosis. Combined, these interactions may control the expression of a negative regulator of RTK/MAPK signaling which in turn regulates the ability of Hid to induce apoptosis through MAPK-dependent phosphorylation. We cannot exclude that Kdm5 also regulates the expression of a specific apoptotic gene. Max is a transcriptional co-activator of dMyc. Dp is a transcriptional co-activator of E2F1. Question marks (?) indicate uncertainty.

induced apoptosis (Figure 5) and thus may act on the promoter region of the same genes. However, while E2F1 has been shown to be present at the promoter region of endogenous *hid* (Moon et al., 2005), it is unlikely that the cooperative control by Rbf and dMyc occurs on the endogenous *hid* gene. First, all our assays were performed with the heterologous *GMR* promoter which is not controlled by Rbf and dMyc. Second, the *GMR* promoter is not affected by *Kdm5* (Figure 4). Third, expression of endogenous *hid* is not altered in *Kdm5* mutant clones (Table 1). These considerations suggest that Kdm5 regulates

the expression of another gene(s) for the control of the apoptotic activity of Hid.

While we cannot exclude that another apoptotic gene is regulated by Kdm5, E2F1 and dMyc, our genetic analysis points towards control of MAPK activity (Figure 6). We showed previously that in contrast to Reaper and Grim, Hid is negatively controlled by phosphorylation of five Ser or Thr residues by Erk-type MAPK (Bergmann et al., 1998). A phosphorylation-defective mutant of Hid under GMR-control (*GMR-hid*^{Ala5}) cannot be suppressed by *Kdm5* mosaics (Figure 6) strongly suggesting that Kdm5 controls MAPK phosphorylation activity for regulation of Hid. This may occur through regulation of a gene which directly or indirectly controls MAPK activity (Figure 7). Regulation of MAPK activity by Kdm5 is not unprecedented. Drelon et al. (2019) showed that Kdm5 regulates MAPK activity through transcriptional control of the receptor tyrosine kinase (RTK) *torso* in the prothoracic gland (Drelon et al., 2019). While there is no known role of Torso for development of imaginal discs in *Drosophila* larvae, other RTKs such as the *Drosophila* EGFR receptor (DER) have very established roles, especially for eye development (Malartre, 2016). However, while Drelon et al. (2019) demonstrated that Kdm5 is required for MAPK activity in prothoracic glands, our data suggest that Kdm5 negatively controls MAPK activity for the control of Hid-induced apoptosis in imaginal discs. This discrepancy may be caused by tissue-specific differences for the control of MAPK activity by Kdm5 and may be the result of the control of different target genes in these tissues. Therefore, instead of *torso* in the prothoracic gland, genes that negatively control DER and thus MAPK activity such as *argos* or *sprouty* may be controlled by *Kdm5* for control of Hid-induced apoptosis. Future work will reveal the target gene(s) of Kdm5 that control MAPK activity for regulation of Hid-induced apoptosis in *Drosophila*.

Kdm5 is not the only JmjC-domain-containing protein that regulates Hid activity. The *Drosophila* ortholog of the phosphatidylserine receptor, dPSR, has also been implicated in controlling Hid activity (Krieser et al., 2007). dPSR is a JmjC-domain-only protein, and its mammalian ortholog JMJD6 demethylates histone H3 at Arg2 and histone H4 at Arg3 (Chang et al., 2007). Initially, PSR was classified as the phosphatidylserine receptor for engulfment of apoptotic cells (Fadok et al., 2000). However, the function of dPSR in engulfment in *Drosophila* is uncertain. *dPSR* mutant macrophages (hemocytes) can engulf apoptotic cells and dPSR protein is nuclear (Krieser et al., 2007) which is inconsistent with the predicted localization of an engulfment receptor at the cell surface. Interestingly, further characterization of dPSR revealed that it inhibits the ability of Hid to induce apoptosis (Krieser et al., 2007) suggesting that Kdm5 and dPSR may have antagonistic activities for the control of the apoptotic activity of Hid. Whether Kdm5 and dPSR directly interact to exert this antagonistic function is unknown. It is also unknown whether dPSR requires a functional JmjC domain for this activity.

Given the irreversible effect of apoptosis, it is not surprising that apoptosis is a highly regulated process. Apoptosis is controlled at transcriptional, post-transcriptional (alternative splicing) and post-translational (phosphorylation, ubiquitylation, etc.) levels. Here, we also add epigenetic control as an additional control mechanism of apoptosis. Resistance to apoptosis is a hallmark of many cancers and is often accompanied by epigenetic deregulation (Chakraborty et al., 2024; Ozyerli-Goknar and Bagci-Onder, 2021). Thus, it is crucial to better understand the epigenetic mechanisms that suppress apoptosis in various cancers and other diseases to help advance the development of targeted therapies.

We have performed recessive mutagenesis screens for the control of Hid-induced apoptosis in genetic mosaics using *GheF* screening for all autosomal chromosome arms. These screens yielded mutations in the core components of the apoptotic pathway, Dronc, Dark and Drice (Wu et al., 2016; Xu et al., 2005; Xu et al., 2006; Xu et al., 2009). In addition, we recovered mutations in genes that modify the outcome of the apoptotic pathway such as *Uba1* affecting ubiquitylation and *D-cbl*, regulating MAPK activity (Lee et al., 2008; Wang et al., 2008). We also recovered mutations in *vps25*, *cos2* and *ptc* which affect apoptosis non-autonomously through control of the Notch and Hedgehog signaling pathways (Christiansen et al., 2012; Christiansen et al., 2013; Herz et al., 2006; Herz et al., 2009). Here, we added epigenetic control of apoptosis by the recovery of *Kdm5* mutants as an additional layer of regulation. In summary, these screens underscore the importance of unbiased mutagenesis screens to understand the control of a biological process, in this case apoptosis, at all possible levels. This detailed understanding will also have important implications for understanding and treatment of diseases in human patients.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

H-MH: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing–review and editing. AB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Resources, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fceld.2024.1471050/full#supplementary-material

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