

Appendix

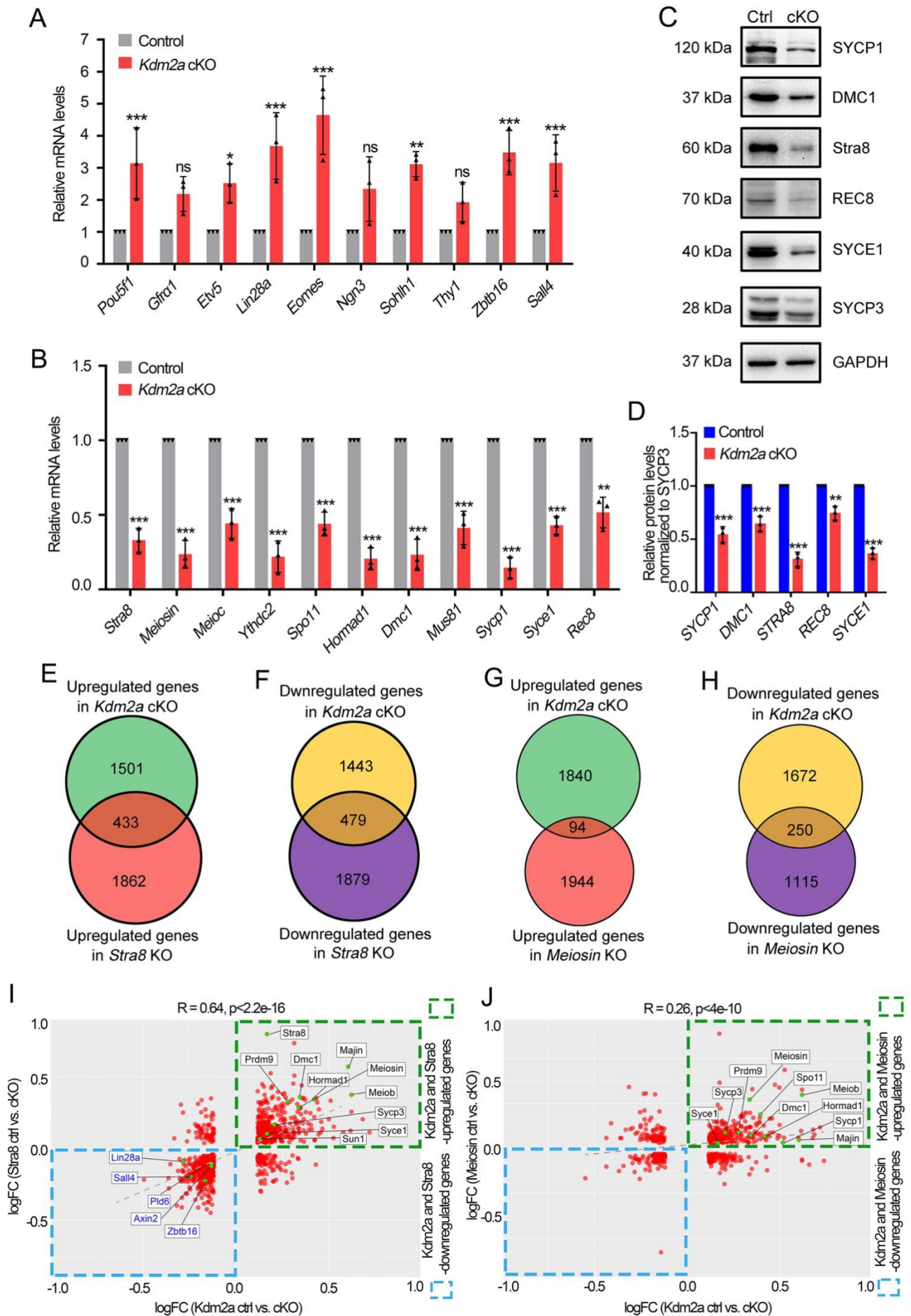
Histone demethylase KDM2A recruits HCFC1 and E2F1 to orchestrate male germ cell meiotic entry and progression

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Appendix Figure S1



Appendix Figure S1. KDM2A regulates gene expression of *Stra8* and *Meiosin*.

(A-B) RT-qPCR analyses the upregulated genes are related to SSC development (A) and the downregulated genes are related to meiosis (B) in *Kdm2a* cKO c-KIT⁺ differentiating spermatogonia cells from RNA-seq data. Data were presented as the mean $\pm$ SD. n = 3. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

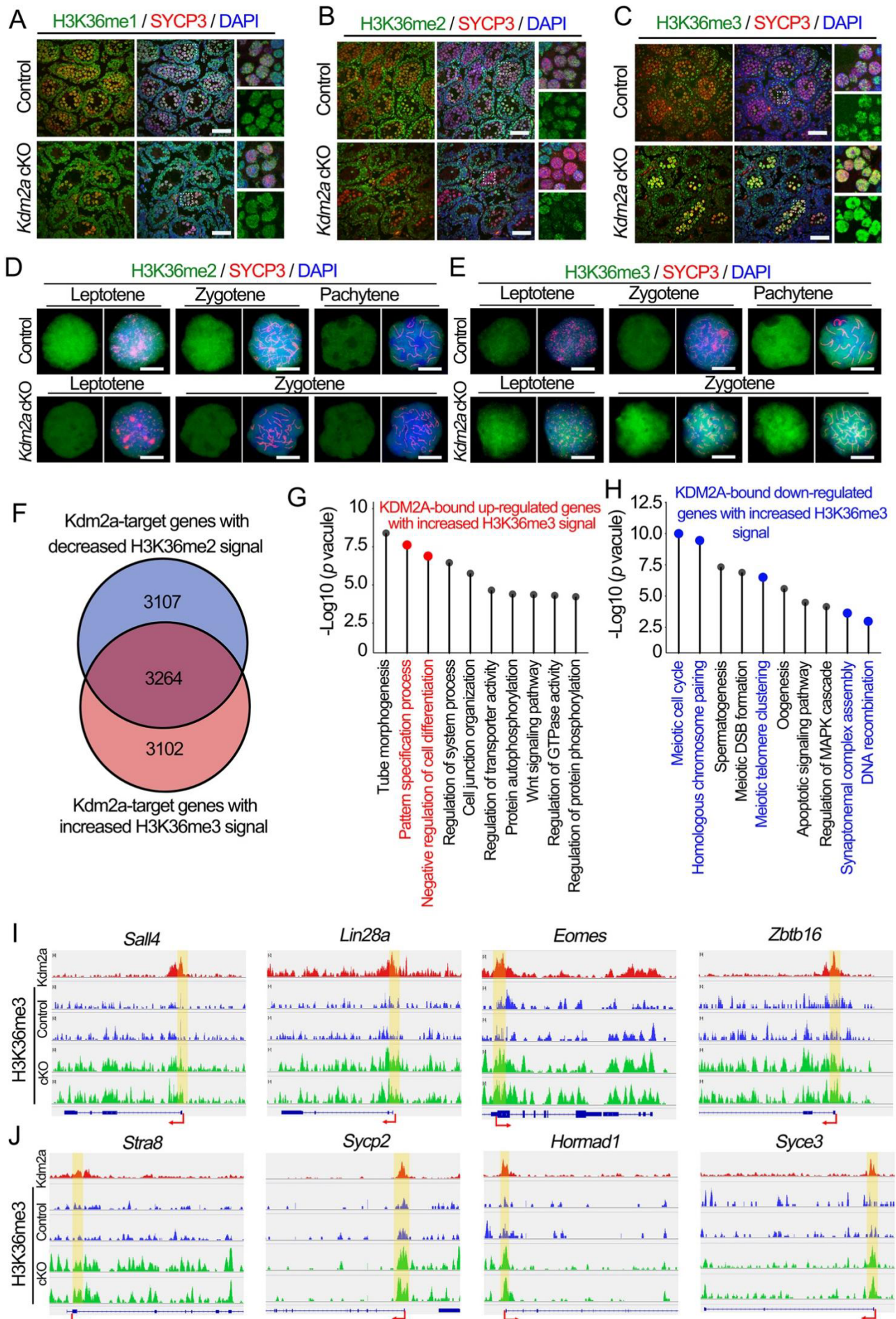
(C-D) WB analysis of protein levels of the selected downregulated genes that are associated with meiosis-related genes in *Kdm2a* cKO c-KIT⁺ cells identified from RNA-seq data. GAPDH served as a loading control. The quantifications were normalized to SYCP3 (D). Data were presented as the mean $\pm$ SD. n = 3. ***P* < 0.01; ****P* < 0.001.

(E-F) Venn diagrams showing the overlap among the upregulated transcripts (E) and downregulated transcripts (F) from our RNA-seq data together with upregulated transcripts (E) and downregulated transcripts (F) from the RNA-seq database after deletion *Stra8*.

(G-H) Venn diagrams showing the overlap among the upregulated genes (G) and downregulated genes (H) from our RNA-seq data together with upregulated genes (G) and downregulated genes (H) from the RNA-seq database after deletion *Meiosin*.

(I-J) Distribution of log (FC, fold change) values for DGEs in *Kdm2a* cKO c-KIT⁺ cells. The genes involved in indicated signaling pathways and co-regulated by *Kdm2a* with *Stra8* (I) and *Meiosin* (J) are marked in red.

Appendix Figure S2



Appendix Figure S2. KDM2A is essential for normal deposition of H3K36me2/3.

(A-C) Co-Immunofluorescent staining of SYCP3 with H3K36me1 (A), H3K36me2 (B), and H3K36me3 (C) on testis sections from control and *Kdm2a* cKO mice at P14, respectively. Nuclei were stained with DAPI. Enlarged images are shown on the right panel. Scale bars = 50 μ m.

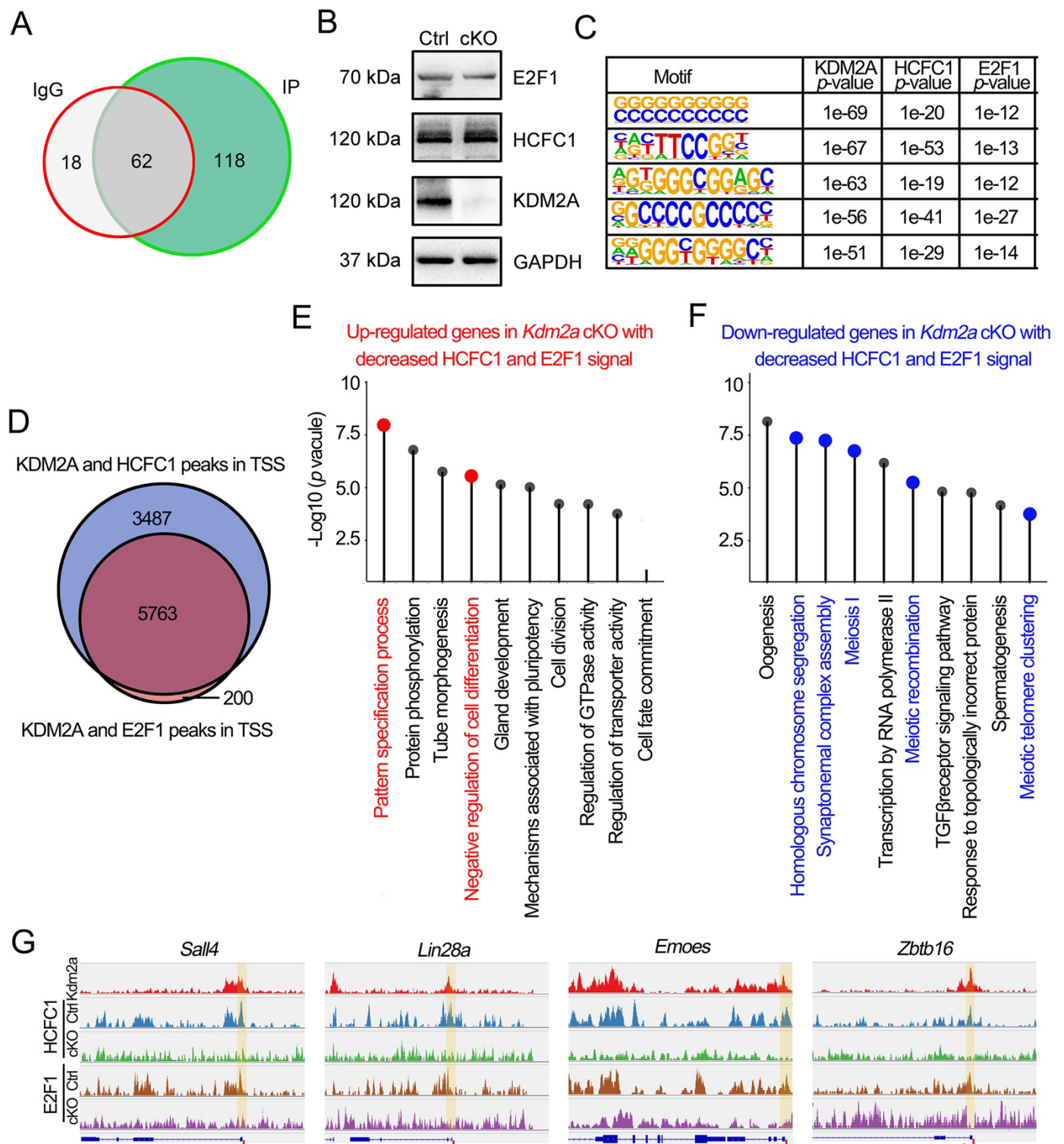
(D-E) Co-Immunofluorescent staining of SYCP3 with H3K36me2 (D) and H3K36me2 (E) on spermatocyte spreads from control and *Kdm2a* cKO testes at P18. Nuclei were stained with DAPI. Scale bars = 5 μ m.

(F) Venn diagrams showing overlap of *Kdm2a*-bound genes with decreased H3K36me2 signal genes (6371), and *Kdm2a*-bound genes with increased H3K36me3 signal genes (6366).

(G-H) GO analyses of the upregulated genes (G) and downregulated genes(H) with increased H3K36me3 signal in *Kdm2a*-bound genes.

(I-J) Genome browser tracks depicting reads accumulation of H3K36me3 on representative genes in control and *Kdm2a* cKO c-KIT-positive cells using IGV software.

Appendix Figure S3



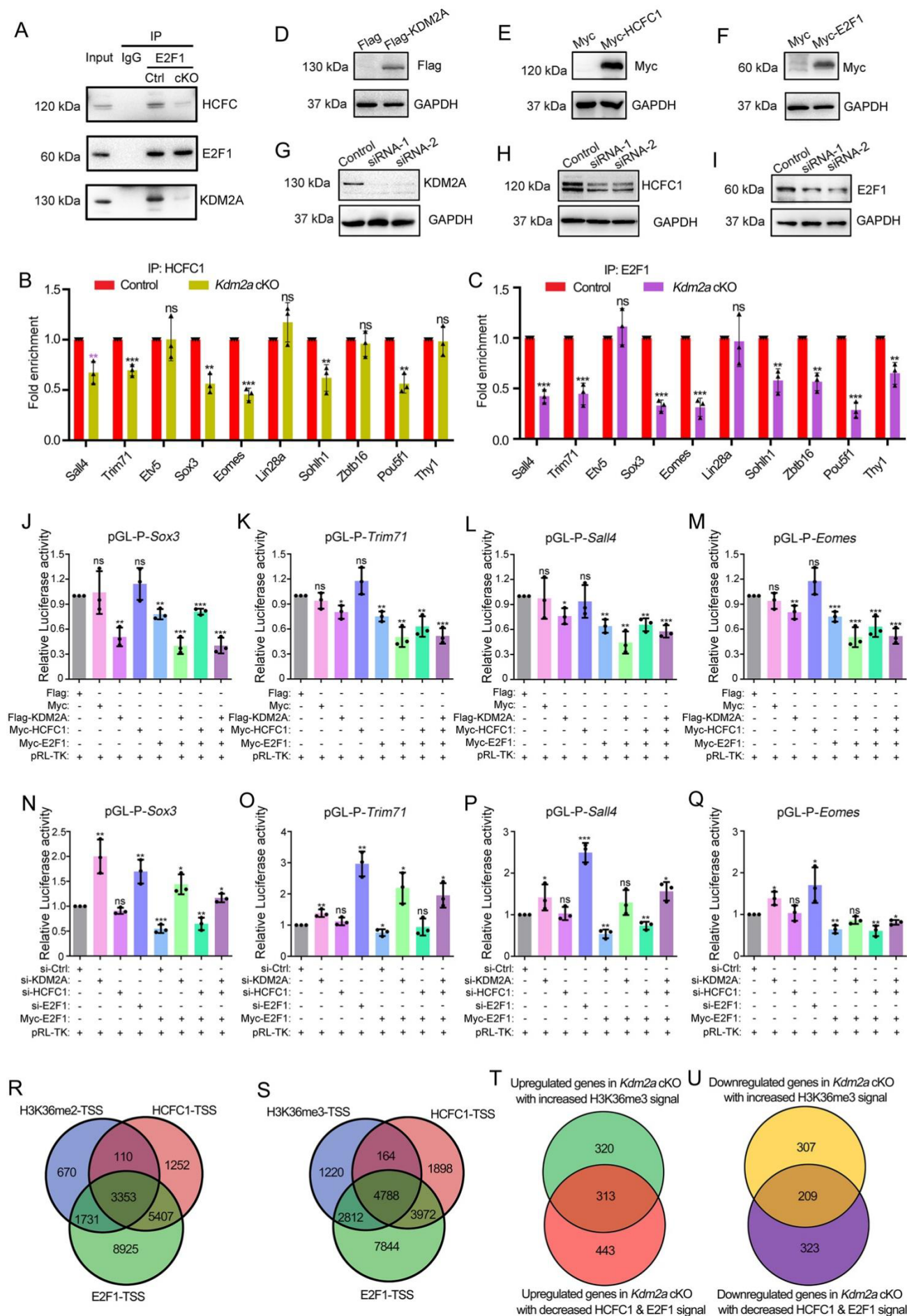
Appendix Figure S3. KDM2A cooperates with HCFC1 and E2F1 in germ cells.

(A) Venn diagrams representing the overlap among KDM2A-interacting partners from IP and IgG by IP-MS data.

(B) The interacting protein (E2F1 and HCFC1) expression levels from control and *Kdm2a* cKO c-KIT⁺ cells were determined by WB. GAPDH was used as a loading control.

- (C) Top five ranked sequence motifs enriched in ChIP-seq data from KDM2A (left), HCFC1 (middle), and E2F1 (right) with E values are shown.
- (D) Venn diagram representing the overlap between HCFC1-bound TSS and E2F- bound TSS from *Kdm2a* cKO c-KIT-positive cells.
- (E) GO analyses of the upregulated genes in *Kdm2a* cKO c-KIT-positive cells with decreased HCFC1 and E2F1 signals.
- (F) GO analyses of the downregulated genes of *Kdm2a* cKO c-KIT-positive cells with decreased HCFC1 and E2F1 signals.
- (G) Genome browser tracks depicting reads accumulation of HCFC1 and E2F1 on representative genes in control and *Kdm2a* cKO c-KIT-positive cells using IGV software.

Appendix Figure S4



Appendix Figure S4. KDM2A and E2F1 regulate the expression of downregulated genes.

(A) IP analysis was performed with anti-E2F1 antibody. The protein levels of immunoprecipitated KDM2A, HCFC1 and E2F1 in WT and *Kdm2a* cKO c-KIT positive cells were checked by WB assay.

(B-C) RIP-qPCR analyses of the association of the selected gene mRNAs with HCFC1(L) and E2F1(M) in control and *Kdm2a* cKO c-KIT-positive cells are shown. Biologically independent mice (n = 3) were examined in three separate experiments. Data were presented as mean $\pm$ SD. ns, not significant, $**P < 0.01$, $***P < 0.001$.

(D-F) Immunoblotting analyses the overexpression of KDM2A (A), HCFC1 (B), and E2F1 (C) were transfected into HEK293T cells, respectively. GAPDH served as a loading control.

(G-I) Immunoblotting analyses the knockdown of KDM2A (E), HCFC1 (F), and E2F1 (G) were transfected into HEK293T cells, respectively. GAPDH served as a loading control.

(J-M) Luciferase reporter assays show that when *Kdm2a* and *E2f1* were overexpressed in different combinations, the luciferase activity of the *Sox3* (N), *Trim71* (O), *Sall4* (P), and *Eomes* (Q) promoter regions in HEK293T cells was significantly increased. Data were presented as mean $\pm$ SD. n = 3. ns, not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(N-Q) Luciferase reporter assays show that when *Kdm2a* and *E2f1* were knocked down in different combinations, the luciferase activity of the *Sox3* (R), *Trim71* (S), *Sall4* (T), and *Eomes* (U) promoter regions in HEK293T cells was remarkable decreased. Data were presented as mean $\pm$ SD. n = 3. ns, not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

(R) Venn diagram showing overlap in the H3K36me2 TSS, HCFC1 TSS, and E2F1 TSS of *Kdm2a* cKO c-KIT-positive cells.

(S) Venn diagram showing overlap in the H3K36me3 TSS, HCFC1 TSS, and E2F1 TSS of *Kdm2a* cKO c-KIT-positive cells.

(T) Venn diagram showing the overlap of upregulated genes with increased H3K36me3 signal and upregulated genes with decreased HCFC1&E2F1 signal in *Kdm2a* cKO c-KIT-positive cells.

(U) Venn diagram showing the overlap of downregulated genes with increased H3K36me3 signal and downregulated genes with decreased HCFC1&E2F1 signal in *Kdm2a* cKO c-KIT-positive cells.

Appendix Table S1.

Primer sequences are used in this study.		
Target	Sequence (5' to 3')	Application
<i>STRA8GFP-cre</i>	F: ACTCCAAGCACTGGGCAGAA	Genotyping
	R1: GCCACCATAGCAGCATCAAA	
	R2: CGTTTACGTCGCCGTCCAG	
<i>KDM2A-loxp</i>	F: CAACCTGACCCTAATTGTACAGC	Genotyping
	R: CCTGGTGTGGCTAAACTCT	
<i>Gapdh</i>	F: AGGTCGGTGTGAACGGATTTG	qPCR
	R: GGGGTCGTTGATGGCAACA	
<i>Stra8</i>	F: AGGCTGTTGGACCAGATGCT	RT-qPCR
	R: CATGTGCAGAGATGATGCTGT	
<i>Stra8</i>	F: CTGCGACTACAGCTATGATCCACC	CHIP-qPCR
	R: AGCCCCTTTCTAAAGTATGCAACAGG	
<i>Meiosin</i>	F: CCCGTGAAGCCCAAAGACAGC	RT-qPCR
	R: GGTCTCCCATCGTCGTCCTCA	
<i>Meiosin</i>	F: ACTTTGTTTGCAGCTACAGGAACTGGCAT	CHIP-qPCR
	R: CTCCTGTGTCCCATCCGACCCTT	
<i>SPO11</i>	F: GATGCAACATTTTCAGCGGCTCC	RT-qPCR
	R: CACATTATCTCGATGCCGTAGGGA	
<i>SPO11</i>	F: TCCCTTCTCCCATACCTATCCCCA	CHIP-qPCR
	R: AGGGGAAATAATACTTGCAAAGCTCT	
<i>SYCP1</i>	F: TCCTTCACAAAATATATCTCGGCTT	RT-qPCR
	R: AACATCAAATTCAAAGGCTGTTT	
<i>SYCP1</i>	F: ATGAAACACTCTATAACACGCACCAG	CHIP-qPCR
	R: GTTCTCTACCAAGTCTGCGCTCA	
<i>Meioc</i>	F: CCCATCTAGAGTTGATCGGTT	RT-qPCR
	R: AACAATTTTCATCCTTTCTACGTGA	
<i>Ythdc2</i>	F: TTCCCTTTCAGTTTGCACACC	RT-qPCR
	R: TGGCATACAACACTTCATCAAGG	
<i>Hormad1</i>	F: CAAAACAAGAAGCGGAAA	RT-qPCR
	R: GCACACCTTCAAATAATACAGC	
<i>Dmc1</i>	F: CATTCTGGCTCACGCTTCCAC	RT-qPCR
	R: TGTACCAATTCCCCACCTACTCC	
<i>Mus81</i>	F: ACACCGTACGCAAGCTACACGTTGGA	RT-qPCR
	R: AAGGCTAAGGTTGTGGACAGACCCAT	
<i>Syce1</i>	F: CCTGCTCCACGGGCCTGA	RT-qPCR
	R: CACTGGCTTTCTTTTTTGGTAAGTCC	
<i>Pou5f1</i>	F: CCAGACCACCATCTGTCGCTTCGAG	RT-qPCR
	R: TCCAGACTCCACCTCACACGGTT	
<i>Gfra1</i>	F: CTGAGAATGAGATTCCCACACACGTT	RT-qPCR
	R: TGAACACCATCACCGGCAGT	
<i>Etv5</i>	F:GGCATGGAATTTAAGCTCATAGAACCGGAA	RT-qPCR
	R:TAAGCAGGGTTGTCTTCAAAGTGAGTCAGA	

<i>Lin28a</i>	F: AGTTCACCTTTAAGAAGTCTGCCAAGGGTC	RT-qPCR
	R: AGCTTGCATTCTTGGCATGATGGTCT	
<i>Eomes</i>	F: ACCTTCTCAGAGACACAGTTCATCGC	RT-qPCR
	R: TTTGAACGCCGTACCGACCTCC	
<i>Ngn3</i>	F: CACTGCTGCTTGACACTGACCC	RT-qPCR
	R: AATTGGAAGTGAAGCACTTCGTGG	
<i>Sohlh1</i>	F: CGGGGACCCTGAATCTTCCGGCAT	RT-qPCR
	R: CAGGGTCTCCGATGAAGCTTGGCTCT	
<i>Thy1</i>	F: CTTGGCACCATGAACCCAG	RT-qPCR
	R: ACGTGCTTCTCTTCTCTCGG	
<i>Zbtb16</i>	F: CAACCGCACCTTCCCCAGCCACACG	RT-qPCR
	R: CCAACTGGTGCTTGAGGCTGAACT	
<i>Sall4</i>	F: CCCCAGCATGCCCTCGGGTA	RT-qPCR
	R: AGCACTCAGCGCGGCCAT	
<i>Meioc</i>	F: ATCTCCCAACCAACCGCTACCAAGCC	ChIP-qPCR
	R: AAGGAGCAGTTAGCCGACTTCGCCCTA	
<i>Dmc1</i>	F: AAAACTATCCACGGGAGATAGGT	ChIP-qPCR
	R: CTGTTTCCTTTTCCCCTTAGATGG	
<i>Cpeb1</i>	F: CTCCTTGAATGTGATTTGGGCACT	ChIP-qPCR
	R: GTCTCAAACAAGATATGCCGCTGA	
<i>Tex15</i>	F: TCAGCCTCTAAAGTGATGAGATTACAAGC	ChIP-qPCR
	R: ATCCTATAAACCTTTCTCTATAGCCAT	
<i>Zfp541</i>	F: AAATAAATAAGCCAGTCCTGCAGTTC	ChIP-qPCR
	R: GGACACCCTCCAGTATAGATCCC	
<i>Stra8</i>	F: AGACAAGGTGGTGAGAACTGAG	pGL4.10 Plasmid construction
	R: ACTTAGCTTTCTCTCGTGTAAGGAC	
<i>Meiosin</i>	F: TGCAGGTCACAAAAGAGAATAAGGAAC	pGL4.10 Plasmid construction
	R: TCCCGACGCGCAGACCGAG	
<i>Spo11</i>	F: ACACCGCAATGCTCACAC	pGL4.10 Plasmid construction
	R: CGAACCTTTCCGCGCTCT	
<i>Sycp1</i>	F: TTATTTTGACATGGCTGCACTCCC	pGL4.10 Plasmid construction
	R: CGGCTCAATTTAACGGTTGGTC	
KDM2A full length	F: ATGGAACCTGAAGAAGAAAGGATTCTG	Overexpression plasmid construction
	R: TTAGCTAATCTTCTGTATCAGTTTCTCAT	
HCFC1 full length	F: ATGGCTTCGGCTGTGTCTCCC	overexpression plasmid construction
	R: TCACTGACCATCAGCCTTAGACT	
E2F1 full length	F: ATGGCCGTAGCCCCCGCG	overexpression plasmid construction
	R: TCAGAAATCCAGAGGGGTCAGGT	
KDM2A-siRNA-1	5'-GAACCCGAAGAAGAAAGGAUUCGUU-3'	knockdown experiment
KDM2A-siRNA-2	5'-GAGGAGGAGCGAGAGAAACUCUAUA-3'	knockdown experiment
E2F1-siRNA-1	5'-UAUCUGUACUACGCAGCUGTT-3'	knockdown experiment
E2F1-siRNA-2	5'-GGACCUUCGUAGCAUUGCATT-3'	knockdown experiment
HCFC1-siRNA-1	5'-CCCUAUCAUCACAGUGCACAATT-3'	knockdown experiment
HCFC1-siRNA-2	5'-GCCCCGAAUGAGAAGGGCUAUTT-3'	knockdown experiment
HCFC1-siRNA-3	5'-GCAACCACCAUCGGAAAUAAATT-3'	knockdown experiment
NC-siRNA	5'-UUCUCCGAACGUGUCACGUTT-3'	knockdown experiment

Appendix Table S2.

Antibodies used in this study.					
Antibodies	Species	Applications	Concentration	Sources	Cat #
DDX4/MVH	Rabbit	IF	1:400	Abcam	ab13840
SYCP3	Rabbit	IF	1:200	Abcam	ab15093
SYCP3	Mouse	IF	1:100	Santa	sc-74569
SYCP3	Mouse	WB	1:100	Santa	sc-74569
rH2AX	Mouse	IF	1:200	Abcam	ab26350
rH2AX	Rabbit	IF	1:200	Abcam	ab11174
PLZF	Mouse	IF	1:100	Santa	SC-28391
STRA8	Rabbit	IF	1:200	Milipore	ABN1656
STRA8	Rabbit	WB	1:2000	Milipore	ABN1656
Rad51	Rabbit	IF	1:400	Abcam	ab133534
c-kit	Goat	IF	1:200	R&D	AF1356
Dmc1	Rabbit	IF	1:200	Proteintech	13714-1-AP
Dmc1	Rabbit	WB	1:2000	Proteintech	13714-1-AP
Rec8	Rabbit	WB	1:1000	Proteintech	10793-1-AP
SYCE1	Rabbit	WB	1:1000	Proteintech	11063-1-AP
SYCP1	Rabbit	IF	1:200	abcam	ab15090
SYCP1	Rabbit	WB	1:1000	ABclonal	A12139
KDM2A	Rabbit	IF	1:1000	abcam	ab191387
KDM2A	Rabbit	WB	1:1000	abcam	ab191387
KDM2A	Rabbit	IP	1:100	Proteintech	24311-1-AP
H3K36me1	Rabbit	IF	1:500	ABclonal	A2364
H3K36me1	Rabbit	WB	1:1000	ABclonal	A5924
H3K36me2	Rabbit	WB	1:1000	ABclonal	A2365
H3K36me2	Rabbit	IP	1:100	Active Motif	39056
H3K36me2	Rabbit	IF	1:200	Active Motif	39056
H3K36me3	Rabbit	IF	1:500	ABclonal	A2366
H3K36me3	Rabbit	WB	1:1000	ABclonal	A2366
H3K36me3	Rabbit	IP	1:100	CST	4909
HCFC1	Rabbit	IP	1:100	CST	69690
HCFC1	Rabbit	WB	1:1000	CST	69690
E2F1	Rabbit	WB	1:1000	Proteintech	66515-1-Ig
E2F1	Rabbit	IP	1:100	Proteintech	66515-1-Ig
GAPDH	Rabbit	WB	1:5000	Proteintech	10494-1-AP
FLAG-tag	Rabbit	IP	1:100	Proteintech	20543-1-AP
Myc-tag	Rabbit	IP	1:50	Proteintech	16286-1-AP
FLAG-tag	Mouse	WB	1:3000	ABclonal	AE005
Myc-tag	Mouse	WB	1:3000	Proteintech	60003-2-Ig
Goat anti-rat IgG	Goat	WB	1:10000	Biosharp	BL002A
Goat anti-rat IgG	Goat	WB	1:10000	Thermo Fisher	62-9520
HRP Goat anti-mouse IgG	Goat	WB	1:10000	Abbkine	A21010-1
HRP Goat anti-rabbit IgG	Goat	WB	1:10000	Abbkine	A21020-1

Dylight 488 Goat anti-mouse IgG	Goat	IF	1:500	Abbkine	A23210
Dylight 594 Goat anti-rabbit IgG	Goat	IF	1:500	Abbkine	A23420
Alexa Fluor™ 488 Goat anti-mouse (H+L)	Goat	IF	1:500	Invitrogen	A11029IgG
Alexa Fluor™ 488 Goat anti-rabbit IgG (H+L)	Goat	IF	1:500	Invitrogen	A32731
Alexa Fluor™ 594 Goat anti-rabbit IgG (H+L)	Goat	IF	1:500	Invitrogen	A11032
Cy3-conjugated Affinipure Goat anti-mouse IgG (H+L)	Goat	IF	1:300	Proteintech	SA00009-1